Exploring Genes

A decade of biomedical genetics

Centre for Biomedical Genetics
1998-2009

Exploring Genes

A decade of biomedical genetics

Centre for Biomedical Genetics
1998-2009

COLOPHON

Projectleader
Annelies Speksnijder
Managing director CBG

Organization
Paul van Laere, Nijmegen

Interviews
Marten Dooper, 's Hertogenbosch
Maarten Evenblij, Amsterdam
Pieter van Megchelen, Leiderdorp

Editing
Maria Hampshire, Glasgow

Design
I am, Annemarike Pieterse, Amsterdam

Photography
Ben Balster, Arnhem

Printer
Ecodrukkers, Nieuwkoop

ISBN/EAN
978-90-815142-1-7

Stratenum 3.223
Universiteitsweg 100
3584 CG Utrecht
The Netherlands
www.biomedicalgenetics.nl

CONTENT

	Preface	4
Agami	Cancer due to disruption of subtle balances	6
Bernards	Molecular diagnostics and personalised medicine – the next great thing	10
Berns	Detecting oncogenes in mouse models	14
Borst	*Trypanosoma* – a soldier in the war on cancer	18
Bos	Spatiotemporal dynamics of signal transduction networks	22
Burgering	Reactive oxygen species – the good, the bad and the ugly	26
Clevers	Identifying (cancer) stem cells	30
ten Dijke	The critical component in a signalling pathway	34
van der Eb	Viral proteins as tools to investigate transformation and signalling	38
Geuze	*Where* do things happen in the cell?	42
Grosveld	Driven by curiosity – and the urge to cure haemoglobinopathy	46
Heck	Global view of the 'cellular society'	50
Hoeijmakers	Understanding ageing from a DNA perspective	54
Kaptein	Adding more cogs to the machine	58
van Lohuizen	A major switch in cell differentiation	62
Moolenaar	The role of lipids in carcinogenesis	66
Neefjes	Networks across the disciplines	70
van Ommen	The freedom to be the first	74
Oostra	Towards a cure for monogenetic mental retardation	78
Sixma	Enzymes at work – see them with your own eyes	82
Verrijzer	Accessing the libraries of life	86
van der Vliet	A very fruitful approach	90
	Facts and figures 1998-2009	94

PREFACE

The CBG provides each of the participating groups with funds for innovations and rapid responses to new technological developments and, importantly, it generates expertise and provides facilities that are accessible for a broader group of researchers in the Netherlands.

In recent decades we have witnessed a revolution in biology and biomedical research. Due to technological innovations in that time, the speed of such research has increased dramatically and there are no signs that it will slow down in the near future. Being involved in this revolution is an enormous privilege.

In the Netherlands, the **Centre for Biomedical Genetics (CBG)** has been a key player in these developments during the past 12 years. The CBG comprises a selected group of internationally recognised researchers from Amsterdam, Utrecht, Rotterdam and Leiden that aim to understand the function of genes and gene products in relation to disease by employing a multidisciplinary approach. It was established in 1998 by the Minister of Education, Culture and Science as one of six Top Research Schools – the only one in the biomedical sciences – within the 'Bonus Incentive Scheme' (or 'In-Depth' Strategy) that was established to promote quality, selectivity and collaboration in Dutch research. The CBG provides each of the participating groups with funds for innovations and rapid responses to new technological developments and, importantly, it generates expertise and provides facilities that are accessible for a broader group of researchers in the Netherlands.

The Centre is very active internally, yet publicity about it has remained low key, because its main assets are the numerous participating groups that function within their own institutions. However the significance of the CBG to these researchers and their institutions and to biomedical sciences in the Netherlands at large has been and still is enormous.

This book provides an update on the activities of the participating groups over the previous decade and gives some idea of the past and current impact of the CBG. These descriptions clearly show how the CBG has been and continues to be a great success – not only because of its scientific output, but also because it has created a scientific community of leading researchers in the Netherlands that gets things done … Together.

Hans Bos
Scientific director

REUVEN AGAMI, THE NETHERLANDS CANCER INSTITUTE AMSTERDAM

CV

Reuven Agami was born in Israel in 1965 and raised there, receiving his bachelor's degree from Tel-Aviv University in 1991. Next he joined the Weizman Institute in Rehovot, where he received his PhD degree for his studies on the Abl kinase and a novel apoptosis pathway in 1998. He then moved to Amsterdam in 1998 where he spent a three-year postdoctoral period at the Netherlands Cancer Institute, supported by an EMBO Long-Term Fellowship. Subsequently, he became an independent group leader at the Netherlands Cancer Institute in 2001, and head of the Division of Gene Regulation since 2008. He was appointed full professor of genetics and pathology at the Erasmus Medical Center in Rotterdam in 2009. Reuven Agami obtained several awards and honors, including the Netherlands Cancer Institute (AVL) prize in 2001, the Dr. Joseph Steiner Prize in 2007 and an elected member of EMBO. Recently, he was selected to receive the 2010 ESCI award and elected editorial board member of EMBO Journal and EMBO Reports.

Cancer due to disruption of subtle balances

Reuven Agami dives deep into the fundamentals of the regulation of gene expression. He studies microRNAs – genes encoding for very small RNAs which control protein production in the cell. MicroRNAs (miRNAs) determine cell activities and properties like division and motility, and are involved in normal differentiation and disease processes (such as cancer). Agami developed genomic-based assays in order to determine the function of miRNAs in tumours. In doing so, Agami has entered the unknown and very complicated territory of the equilibriums between RNA, protein and gene expression that maintain the crucial functions of cells and prevent or stimulate disease states. The focus of his studies is the transition between normal cell behaviour and what happens in cancer.

The first miRNA was discovered in 1993 using the worm *Caenorhabditis elegans*. Even though this turned out to be a real breakthrough, it was followed by seven years of scientific silence. Then, in 2000, a second miRNA from a worm model was identified, and that was the turning point. Obvious evolutionary conservation between worms and mammals suggested the existence of similar genes with similar functions in humans. 'Since then, literally 10,000 publications have been written and more than 1000 different miRNAs are known about today', says Agami, molecular biologist and head of the division Gene Regulation at the Netherlands Cancer Institute (NKI). 'The miRNAs bind to non-coding regions of messenger RNAs (mRNAs) to regulate the rate of protein production. They influence the active genome in cells like stem cells, epithelial cells and

CBG group leader since 2009

Goals

To elucidate the regulation of gene expression by small RNAs and RNA-binding proteins and its implication in cancer initiation and progression.

Facilities and expertise

Facilities: high throughput sequencing machine, flow cytometry, miRNA and mRNA expression platforms, RNA interference libraries for human cells in the form of oligos and vectors, robotics for large scale experiments.
Expertise: functional genetics, RNAi, small RNA molecules non-coding RNAs, RNA binding proteins, oncogenes and tumour suppressor genes, DNA damage pathways.

brain cells. It is increasingly clear that miRNAs act like major switches, directing and maintaining cell identity, and malfunctions of miRNAs are associated with diseases such as cancer.'

Cancer and miRNAs

These miRNAs are widely spread in nature and seem to be part of almost every cell process – including determining the colour of a flower, for instance. Agami and his group are mainly interested in how they influence the rise of cancer and its tumourigenic qualities, cell division, cell differentiation and metastasis. To find out more, the group has developed large expression libraries of known miRNAs, RNA-binding proteins and interference RNAs (iRNAs – which are gene silencers). Then they studied their ability to enhance or inhibit different tumour properties of cells. In doing so, they elucidated the involvement of several miRNAs in the disease processes behind human testicular germ cell tumours, brain tumours and colon cancer.

'We found specific miRNAs that can drive benign tumours into metastasis.'

At the moment Agami's group is focused on the importance of miRNA regulation during development and during cancer. Let-7 is a good example. Let-7 is an miRNA that is expressed in differentiated tissue cells but not in embryonic stem cells; cancer stem cells, for instance, have mostly lost their Let-7 activity. It seems that Let-7 miRNA plays a major part in the transition of stem cells into tissue cells. In somatic (differentiated) cells, Let-7 is produced as a long precursor RNA that is processed (cut) to the mature active form. Agami explains: 'Although there is no Let-7 expression in stem cells, the gene for Let-7 is constantly transcribed. But the Let-7 is not cut out of that large piece of RNA. An RNA-binding protein called Lin-28 binds to the large RNA molecule in the nucleus and prevents the excision of the Let-7 fragment. Therefore it cannot play its major role in the cytosol of cells.'

Subtle interplay of miRNAs

So miRNA molecules do not act alone. For

REUVEN AGAMI, THE NETHERLANDS CANCER INSTITUTE AMSTERDAM

Key publications

1 Agami , R., Blandino, G. et al. (1999). Interaction of c-Abl and p73a and their collaboration to induce apoptosis. Nature, 399, 809-813.

2 Agami R, Bernards R. (2000) Distinct initiation and maintenance mechanisms cooperate to induce G1 cell cycle arrest in response to DNA damage. Cell. Jul 7;102(1):55-66.

3 Brummelkamp, T. R., Bernards, R. and Agami , R. (2002). A system for stable expression of short interfering RNAs in mammalian cells. Science, 295.

4 Brummelkamp, T. R., Bernards, R. and Agami , R. (2002). Stable suppression of tumorigenicity by virus-mediated RNA interference. Cancer Cell, 2, 243-247.

5 Duursma A, and Agami R, (2005). p53-dependent regulation of Cdc6 protein stability controls cellular proliferation. Mol Cell Biol. 2005 Aug;25(16):6937-47.

6 Kolfschoten I, Leeuwen B, et al (2005). A genetic screen identifies PITX1 as a suppressor of RAS activity and tumorigenicity. Cell. 121(6):849-858.

7 Voorhoeve P. M le Sage C. , et al. (2006). A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. Cell. 124(6): 1169-1181.

8 Le Sage, C., Nagel, R., et al (2007). Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. EMBO J 26, 3699-3708.

9 Kedde, M., Strasser, M. J., et al (2007). RNA-binding protein Dnd1 inhibits microRNA access to target mRNA. Cell 131, 1273-1286.

10 Huang, Q., Gumireddy, K., et al (2008). The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. Nat Cell Biol 10, 202-210.

instance, RNA-binding proteins are important for the production of miRNAs and their correct localisation in the cell, as well as for miRNA activity. Thus there is subtle interplay between the binding proteins and miRNAs. As Agami puts it: 'We have learned that such balances between stimulating and inhibiting factors are crucial to a stable expression of genes. We are now investigating the mechanism behind the metastatic characteristics of cells. In these cells we study the interplay between miRNAs, binding of RNA-binding proteins and target genes. We believe that those factors are essential for tumour initiation, maintenance and aggressiveness. Elucidating their role will enable us to better understand the tumourigenic process and to develop new technologies to fight cancer.'

To this end, Agami and his colleagues tested, one by one, miRNAs from their library to see which induced cell invasion and metastasis of benign tumour types. This tedious and laborious work is an unavoidable part of this kind of research. 'We found specific miRNAs that can drive benign tumours into metastasis. Using cellular markers such as luciferase, we were able to follow the development of the metastasis in mouse models. In humans, the identified miRNAs were associated with aggressive lung and breast cancers. Since the identified miRNAs cause metastasis in certain types of tumours, inhibition of their activity may open new avenues for future therapy.'

Inhibition or generation of tumours

Agami's research is fundamental to our understanding of the regulation of genes. It is exciting that some developments may lead to other applications of this new knowledge. 'The activity of miRNA, as well as that of RNA-binding proteins, can be modulated in cells and organisms. There have been inspiring experiments in mice that indicated the remission of tumours due to a specific tumour-toxic miRNA that was delivered by a viral vector and injected into their bloodstream. Unfortunately we showed that the same miRNA, introduced into another environment, actually caused cancer (specifically glioblastomas in the brain); other miRNAs should be tested like this. It was shown recently that injecting monkeys

Current group members

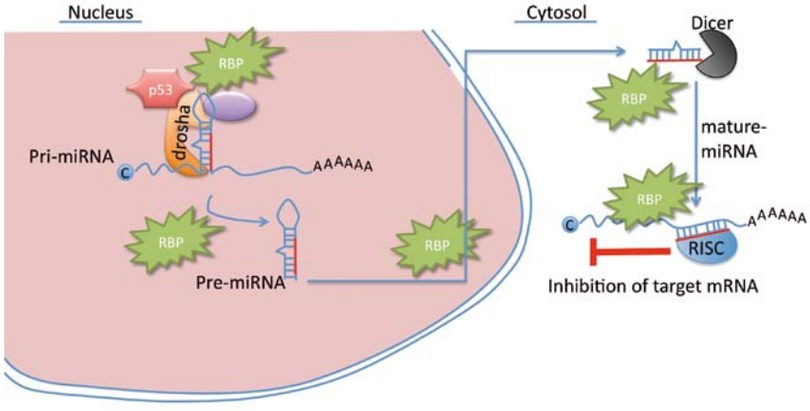
Postdocs
Eitan Zlotorynski
Rani Elkon
Nicolas Léveillé
Gijs van Haaften
Maria Gomez Benito
Henning Schaefer MD
Carlos le Sage
Martijn Kedde
Mathias Jenal

PhD students
Jarno Drost
Marieke van Kouwenhove
Arnold Bos
Carlos Melo

Technicians
Mariette Schrier
Joachim Oude Vrielink

Agami's approach demands huge investments. Numerous compounds need to be tested for their tumour-generating or tumour-inhibiting qualities and fast sequencing of DNA and RNA is crucial to such investigations.

Interplay between RNA-Binding Proteins (RBPs) and miRNAs in cancer



A schematic illustration of the processing pathway of microRNAs (miRNAs). This pathway is controlled and regulated by a number of protein complexes, such as p53, DICER, and RISC. In addition, many RNA binding proteins (RBP) control this pathway at almost every step of the miRNA biogenesis pathway.

with a liver-specific miRNA inhibitor blocked miRNA function and resulted in low cholesterol and resistance to infection to hepatitis C virus. This demonstrated that treatment of mammals with RNA oligonucleotides can influence the course of disease. It remains to be shown whether this type of treatment is also beneficial in the case of human cancer and metastasis.'

Funding through CBG is important for Agami's investigations because his research is very risky and the results of his projects are not predictable. 'There are few funds that you may use at will. But this is the best way to operate in my field of research. We really stand here at the front of science. There are no fence posts for guiding our way. There are no guarantees of success. These are trial and error tests.' Agami's approach simply demands huge investments. Numerous compounds need to be tested for their tumour-generating or tumour-inhibiting qualities and fast sequencing of DNA and RNA is crucial to such investigations. 'For that purpose and with the help of CBG we have purchased a high-throughput facility with robots and a sequencer that has the capacity to analyse the human genome in just a few days. Many groups within the NKI benefit from these facilities.'

RENÉ BERNARDS, THE NETHERLANDS CANCER INSTITUTE AMSTERDAM

CBG group leader since 1998

Molecular diagnostics and personalis

Around the time the CBG began, in 1998, microarray technology emerged in the scientific community, bringing with it the potential to survey the activity of all 25,000 genes in a single experiment, and providing unprecedented new insights into the relationships between cell behaviour and gene expression patterns. As René Bernards recalls: 'We therefore decided to invest a significant part of the CBG grant in a central microarray facility, which was also available for other research groups'. In retrospect this was an excellent choice because it was crucial to the birth of 'MammaPrint'. Bernards continues: 'It is fair to say that without that initial funding for the microarray facility, MammaPrint would not be here today.'

MammaPrint is Bernards' baby. Bernards is the head of the Division of Molecular Carcinogenesis at the Netherlands Cancer Institute (NKI). He and his co-workers conceived of the idea to make a genomic scan of breast tumour tissue to determine the aggressiveness of the tumour. Based on the screening of the expression of all 25,000 genes, they focused on a set of 70 that accurately selects which patients are likely to benefit from treatment with chemotherapy and which are likely to be harmed by it (because they suffer from adverse effects without any beneficial effects of the drugs).

Microarray technology for breast cancer prognosis

Bernards explains: 'We started the microarray facility, but we experienced technical difficulties in the accomplishment of our ideas. It turned out that the technology that was publicly available at the time was not sophisticated enough for our

CV

René Bernards received his PhD degree in 1984 from the University of Leiden. He worked as a postdoc in the lab of Robert Weinberg at the Whitehead Institute in Cambridge, USA. In 1988 he continued his research on genes involved in cancer as an assistant professor at the Massachusetts General Hospital. In 1992 he returned to the Netherlands as a staff member at the Netherlands Cancer Institute in Amsterdam. Since 1994 he also is part-time professor at the University of Utrecht. In 1998 he setup with CBG funds a microarray facility, in which he developed, amongst others, the MammaPrint breast cancer test. This test predicts disease progression in breast cancer and is made available worldwide by Agendia, a company that he co-founded in 2003. His current research focuses on genes that cause resistance against cancer drugs.

Bernards has received several awards for his work, including the Pezcoller Foundation-FECS Recognition for Contribution to Oncology, the ESMO Lifetime Achievement Award in Translational Research in Breast Cancer and the Spinoza Prize from NWO. He also is a member of the Royal Netherlands Academy of Sciences.

Goals

In the period 1998-2003 the goal was to understand gene expression in cancer. This has led to new types of molecular diagnostic tests that are already in clinical use. From 2003 the goal is to understand resistance against cancer drugs, by using innovative genetic screens.

Facilities and expertise

The group developed a DNA microarray facility to study gene expression in cancer, and also large scale RNA interference screens to study the function of genes in cancer.

ed medicine – the next great thing

purposes, so we sought cooperation with the American based company, Rosetta Inpharmatics Inc., which was started by a good personal friend of mine. They had developed an inkjet-based printer technology to produce microarrays and with their technology we were successful in establishing the MammaPrint breast cancer prognosis signature. MammaPrint has become a success story and Agendia, a spin-off company of NKI, has developed the gene test into a commercial product, which is in routine clinical use throughout the world today. The prognostic importance of the MammaPrint is not in debate. The test is applied in the clinic and healthcare providers such as Medicare in the USA reimburse the costs. Dutch health insurance will probably do the same in the near future.'

'The CBG grant enabled us to generate a collection of 24,000 shRNA vectors. Now we can switch off nearly every gene in the human genome.'

Gene silencing achieved with RNA vectors

Five years later the next CBG grant was awarded, in 2003. This was the era of the newly discovered RNA interference (RNAi) technology, which 'silences' genes by using short duplex RNA molecules named siRNAs. Unfortunately the lifetime of an siRNA is less than a handful of days, too short to answer many research questions. In 2002, Bernards' research group developed a plasmid with a gene-construct that can be introduced into cells, which constantly produces so-called 'short hairpin RNA' (or shRNA). This shRNA is processed inside the cell into an siRNA, which in turn can act as a silencer of a chosen gene. Bernards says: 'With the plasmid we developed, which we modestly named "pSUPER", we could in principle silence

RENÉ BERNARDS, THE NETHERLANDS CANCER INSTITUTE AMSTERDAM

Key publications

1 Agami, R., and Bernards, R. (2000). Distinct initiation and maintenance mechanisms cooperate to induce G1 cell cycle arrest in response to DNA damage. Cell 102, 55-66.

2 Brummelkamp, T.R., Bernards, R., and Agami, R. (2002). A system for stable expression of short interfering RNAs in mammalian cells. Science 296, 550-553.

3 Van de Vijver, M.J., He, et al (2002). A gene expression signature as a predictor of survival in breast cancer. New England J. Med. 347, 1999-2009.

4 Brummelkamp, T.R., Nijman, S.M.B., et al (2003). Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-kB, Nature, 424, 797-801.

5 Berns, K., Hijmans, E.M., et al (2004). A large-scale RNAi screen in human cells identifies new components of the p53 pathway. Nature, 428, 431-437.

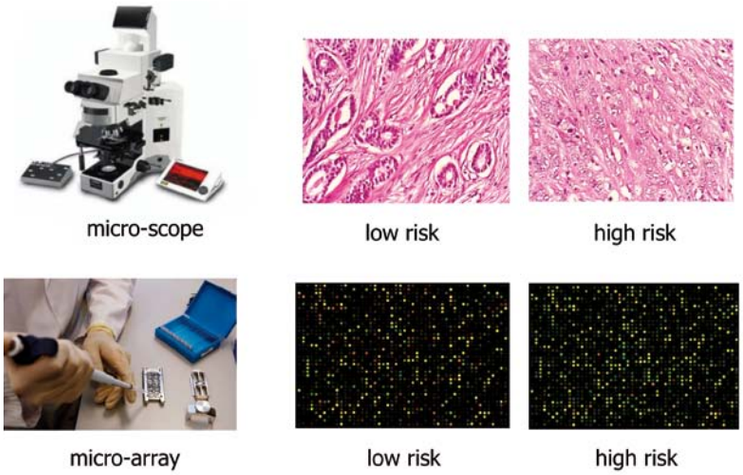
6 Epping, M.T., Wang, L, et al (2005). The human tumor antigen PRAME is a dominant repressor of retinoic acid receptor signaling. Cell, 122, 835-847.

7 Kortlever, R.M., Higgins, P.J., Bernards, R. (2006). Plasminogen activator inhibitor-1 is a critical downstream target of p53 in the induction of replicative senescence. Nature Cell Biol. 8, 878-884.

8 Berns, K., Horlings, H., et al (2007). A functional genetic approach identifies the PI3K pathway as a major determinant of Trastuzumab resistance in breast cancer. Cancer Cell 12, 395-402.

9 Van 't Veer, L.J., and Bernards, R (2008). Enabling personalized cancer medicine through analysis of gene expression patterns. Nature, 452, 564-570.

10 Huang, S., Laoukili, J., et al (2009). ZNF423 Is Critically Required for Retinoic Acid-Induced Differentiation and Is a Marker of Neuroblastoma Outcome. Cancer Cell. 15, 328-340.



Conventional diagnostics rely heavily on the morphology (appearance) of the tumour as judged by microscopic examination. New molecular diagnostic approaches determine the pattern of gene activity in the tumour to assess tumour cell characteristics. The group of Bernards developed a 70- gene test (MammaPrint) that can be used to identify patients with early stage breast cancer that have a high chance of recurrence of the disease. It has been developed into a test that is available for breast cancer patients world wide through a spin off company from CBG named Agendia.

any gene in the human genome. This would be very powerful to study the function of genes.' After all, the best way to detect the function of a gene is to silence it and study what happens to a cell that no longer has the product of that gene. The challenge was to make a very large collection of pSUPER shRNA vectors to 'silence' a significant number of human genes. Bernards continues: 'The costs of such a project were well beyond what a single group could bear, so yet again the CBG funding was instrumental in making a quantum leap forward – the CBG grant enabled us to generate a collection of 24,000 shRNA vectors. Now we can switch off nearly every gene in the human genome. We can also use the technique to silence genes in almost every type of cell, unlike in the past when we had to create and breed knockout mice in order to get tissue in which selected genes are switched off.'

'This tool has been fundamental for a number of publications in top scientific journals like *Nature* and *Cell*', recalls Bernards proudly. 'At the moment we are focusing on how cancer cells become resistant to chemotherapy. In this line of research, cancer cells are grown in combination with drugs, and they normally die. But if we add our library of shRNAs to the cells some of them will survive. Thus the specific shRNA blocks a mechanism that has a

Current group members

Staff
Katrien Berns

Postdocs
Annette Dirac
Michael Hölzel
Sidong Huang
Prasanth Kumar
Michiel van der Heijden
Ian Majewski
Jasper Mullenders

Clinical fellow
Rianne Oosterkamp

PhD students
Ernst Geutjes
Guus Heynen

Technical staff
Marielle Hijmans

connection to the resistance to the drug.' It has been shown in laboratory experiments that eliminating a single gene can influence the sensitivity of a cell for a cancer drug. 'With that kind of experiment we identified a gene called *PTEN* as being involved in sensitivity to a well-known breast cancer drug, trastuzumab (Herceptin®). We have confirmed the relevance of the PTEN signalling pathway as a determinant of trastuzumab resistance in 50 clinical breast cancer samples. We have recently used the same approach to identify mechanisms of resistance to the drug retinoic acid in children with neuroblastoma.'

Kinase gene mutations and cancer drug responses

The use of large-scale gene silencing is now used widely. 'We have made several further investments with CBG money to perform such shRNA screens', says Bernards. 'This has resulted in cooperation between different CBG research groups. There has been a lot of innovation going on, thanks to CBG. The good thing about this kind of grant is that there is no big discussion about the way in which it is spent. This field of research changes so quickly that it is impossible to determine your research programme for the next 5 years.' But Bernards does not mind looking to the future for the next (third) round of CBG funding. Personalised medicine is the topic this time. 'The relationship between mutations in genes and reaction to drugs is a huge issue. It is interesting that today most of the genes involved in modulating drug responses are kinases. These kinase enzymes, of which more than 500 different types are known, connect a phosphate group to a protein, and that plays an important role in the transfer of signals within cells and between them. With the help of new turbo-sequencing techniques it is possible to analyse the different kinases in the genome of hundreds of cancer patients. In this way we hope to discover which kind of kinase mutation is seen in what type of cancer and determine how the presence of such mutations correlates with responses to specific cancer drugs. Thus we will enter the exciting new era of molecular diagnostics, which will be the most significant innovation in pathology since the invention of the microscope.'

ANTON BERNS, THE NETHERLANDS CANCER INSTITUTE AMSTERDAM

CBG group leader
since 1998

Detecting oncogenes in mouse models

'The most important thing was the opportunity we had to initiate new projects for which it is difficult to attract funding. The Centre for Biomedical Genetics made it possible to do blue-sky research – moving into new territories. Group leaders in institutes like ours have limited capacity to initiate new research lines because nearly all the funding for research comes from competitive grants that usually require extensive preliminary work.'

Anton Berns, scientific director of the Netherlands Cancer Institute, is outspoken in his praise for the funding of the CBG, of which he is one of the key researchers. He quickly recounts the facilities that were largely purchased with CBG money: the microarray facility, the high-throughput screening and the high-throughput sequencer, the VIS-200 system for detection of bioluminescence in laboratory animals, and the miRNA library. 'We established sophisticated techniques at a time that only a few people could still see their potential.'

As in all basic research, there have been successes and disappointments, admits Berns. 'The development of mice that were genetically modified in components that impact on a critical signalling pathway for cellular development – the

CV

Anton Berns studied biochemistry at the University of Nijmegen where he received his Master degree in 1969 and his PhD in 1972. He did his postdoctoral training in the group of Rudolf Jaenisch at the Salk Institute in La Jolla, CA., where he studied the role of retroviruses in causing lymphomas in mice. In 1976 he returned to the University of Nijmegen where his group explored proviral insertional mutagenesis as a means to identify new oncogenes. In 1985 he became staff scientist at The Netherlands Cancer Institute. Here his group did pioneering work to generate and utilize genetically modified mice as a tool to search for new cancer genes. Themes of his current research are establishing of genotype – phenotype correlations of tumours and the use of high throughput proviral insertional mutagenesis to identify components in signaling pathways relevant for cancer. In 1999, he was appointed as Director of Research of the Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital.

Goals

To identify new oncogenes and tumour suppressor genes and understand their contribution to the tumour phenotype in the context of a complete organism; to build mouse models for cancer that closely mimic the human disease and use these to identify more effective drug intervention strategies.

Facilities and expertise

With the support of CBG we have been able to provide part of the infrastructure for our "mouse clinic", in particular non-invasive imaging equipment. This allows us to run clinical trials in a small scale. Expertise involves all aspects of both germline (ES cell derivation and gene targeting) and somatic (adenoviral and lentiviral gene transfer) modification of mice. This expertise is used to generate state-of-the-art mouse models for cancer.

Wnt pathway – was *not*. The *Frat* family of oncogenes that we discovered, which appeared critical in the development in *Xenopus*, did not seem to have an important function in mice. Inactivation of all three *Frat* genes in mice simultaneously had no apparent influence on the development or other features of our mice. They were very happy without these genes.' Berns, who is also a group leader at the division of Molecular Genetics at the Netherlands Cancer Institute (NKI), has focused his research on small-cell lung cancer and mesotheliomas. As a research model Berns uses mice with mutations in several tumour suppressor genes that are also frequently mutated in the cognate human tumours.

His group has developed systems that enable researchers to switch multiple oncogenes and tumour suppressor genes on or off within distinct cell types *in vivo*. This permits the induction of highly specific tumours within a narrow time window and allows investigators to correlate specific genetic defects with tumour characteristics. A range of tumour models has been made using this approach. 'The general picture that transpires from these studies', says Berns, 'is that the mouse models show closer resemblance to the human tumours when they share the same mutations. We apply sensitive in-vivo imaging techniques to follow tumour growth and metastasis in animals in real time, allowing us to monitor their response to genetic and pharmacological interventions.' Berns and his co-workers have also uncovered which types of cells can give rise to which cancer types in lungs. They are now in the process of making these mouse models more suitable for intervention testing. Like the lung cancer models, the NKI has also developed mouse models for studying breast cancer and other forms of cancer. These models play an important role in studying

Berns' group has developed systems that enable researchers to switch multiple oncogenes and tumour suppressor genes on or off.

ANTON BERNS, THE NETHERLANDS CANCER INSTITUTE AMSTERDAM

Key publications

1 Jonkers, J., Meuwissen, R. et al (2001). Induction of mammary tumors by somatic mutation of Brca2 and p53. Nature Genetics, 29, 418-25.

2 Mikkers, H., Allen, J. et al (2002). High throughput retroviral tagging to identify components of specific signaling pathways. Nature Genetics 32, 153-159.

3 Lyons S.K., Meuwissen, R. et al (2003). The generation of a conditional reporter that enables bioluminescence imaging of Cre/Lox-dependent tumorigenesis in mice. Cancer Res. 63, 7042-6

4 Marino, S., Hoogervoorst, D. et al (2003). Rb and p107 are required for normal cerebellar development and granule cell survival but not for Purkinje cell persistence. Development 130, 3359-3368.

5 Meuwissen, R., Linn, S. et al (2003). Induction of small cell lung cancer by somatic inactivation of both Trp53 and Rb1 in a conditional mouse model. Cancer Cell 4, 181-189.

6 Van Amerongen, R., Nawijn, M. et al (2005). Frat is dispensable for canonical Wnt signaling in mammals. Genes & Development 19, 425-430.

7 Van Amerongen R and Berns A. et al (2006). TXR1-mediated thrombospondin repression: a novel mechanism of resistance to taxanes? Genes & Development 20: 1975-81.

8 Krimpenfort, P., IJpenburg, A. et al (2007). P15Ink4b is a critical tumour suppressor in the absence of p16Ink4a. (2007) Nature, 448, 943-6.

9 Jongsma, J., van Montfort, E. et al (2008). A conditional mouse model for malignant mesothelioma. Cancer Cell, 13: 261-271.

10 Uren, A.G., Kool. J. et al (2008). Large-scale mutagenesis in p19ARF and p53 deficient mice identifies cancer genes and their collaborative networks. Cell, 133, 727-41.

the contribution of DNA-damage (repair) defects (that are often observed in cancer) to drug responses.

Oncogenes and tumour suppressor genes in lymphoma

Different mouse models have been used to search for oncogenes in lymphomas and mammary tumours. A large genetic screen with almost a 1000 lymphomas, induced by infection in newborn mice with murine leukaemia virus, yielded almost 600 potential oncogenes and tumour suppressor genes. Berns states: 'In general, I have a healthy scepticism about massive screening efforts, because what should you do with 600 genes?' He continues: 'I hoped we would find genes that acted in completely new pathways, not found previously, mutated in tumour genesis. But that is not evident from the results. We did find a lot of genes known to be mutated in cancer; genes for apoptosis, for cell death, and for chromatin modification. But the interesting point was that we could see specific combinations of genes that were co-mutated or genes that seemed mutually exclusive. Clearly, some of those genes seemed to work together to form tumours and other genes seemed to be redundant and therefore not found together in the same tumour. This enabled us to construct interaction networks of these genes, even without knowing their exact function in cells.'

'CBG has facilitated our work enormously', states Berns. 'It has operated in a very non-bureaucratic fashion, efficiently – an absolute heaven in comparison with many of the other funding sources of the government, resulting in the most pleasant cooperative network I have ever experienced. It works because just a small number of top scientists created the CBG. Groups based in Amsterdam, Utrecht and Rotterdam took the initiative. When one centre proposed a new member, the participants of the other centres decided yes or no. In order to keep it manageable the number of participants was restricted. Evidently, there was a joint vision about how the scientific research should be conducted. We always look critically to what we do or propose and criticism is appreciated because it only can improve our performance.'

Current group members

Staff
Paul Krimpenfort
Margriet Snoek

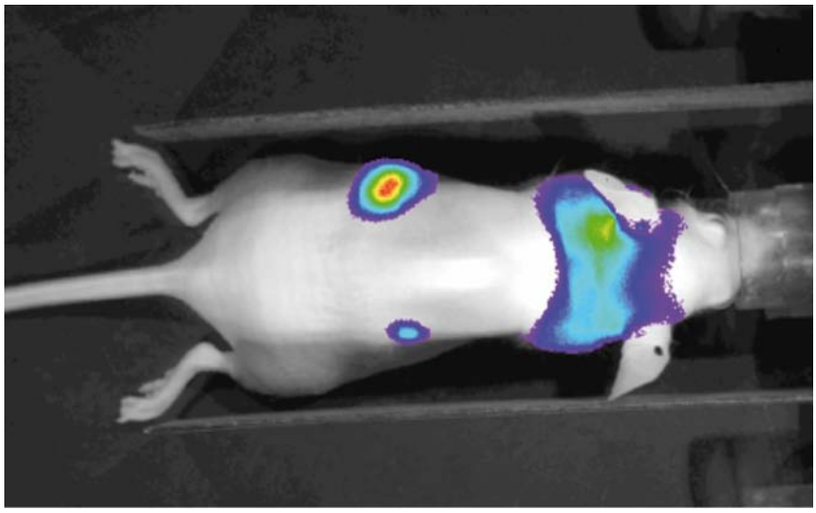
Postdocs
Hilda de Vries
Andor Kranenburg
Min chul Kwon
Kate Sutherland
Anthony Uren

PhD students
Andrej Alendar
Erwin van Montfort

Research assistant
Colin Pritchard

Technicians
Rahmen Bin Ali
Jan Paul Lambooij
Natalie Proost
Johanna Blitz
Fina van de Ahé
John Zevenhoven

'Translating knowledge to clinical application is often problematic. However, in this respect too the CBG has scored rather well.'



Orthotopic grafting of luciferase-labeled small-cell-lung-cancer cell line obtained from a tumour arising after AdenoCre-mediated inactivation of conditional Rb and p53 alleles in lung (via intubation) results in local tumour growth (large signal in thoracic area) as well as metastasis (adrenal gland).

Breast cancer and the MammaPrint

A lot has been learned during the past ten years, Berns concludes. 'But as is the case for most basic research, translating this knowledge to clinical application is often problematic. However, in this respect too the CBG has scored rather well. We have seen important knowledge being implemented in medical practice over these years. The MammaPrint is being increasingly used to evaluate the risk of breast cancer patients to develop recurrent disease and, consequently, to help decide on prescribing adjuvant treatments. But it is disgraceful that while we talk in the Netherlands about translational research and valorisation of knowledge as a top priority, we have been dependent primarily on the United States' healthcare system to bring this 'Dutch' discovery to patients. Rather than embracing such discoveries and promoting their implementation, the company making the MammaPrint would have gone bankrupt if it had depended on reimbursement in the Netherlands. This illustrates how Dutch bureaucracy becomes more and more of an impediment to progress and frustrates scientists, healthcare workers, teachers and small start-up businesses. Unfortunately – and despite all the promises – it is only getting worse.'

PIET BORST, THE NETHERLANDS CANCER INSTITUTE AMSTERDAM

CV

Piet Borst studied medicine in Amsterdam and received his MD, PhD for biochemical studies on mitochondria (supervisor: E.C. Slater). After working on RNA bacteriophages as a postdoc with Weissmann and Ochoa in New York, he returned in 1964 as (associate) professor to Amsterdam University, where he studied a.o. biogenesis of cell organelles (mitochondria, peroxisomes), antigenic variation in African trypanosomes, and multidrug resistance in cancer cells. In 1983 he moved to the NKI-AVL, where he became director of research and chairman of the board of directors, but he retained a part-time university position as Professor of Clinical Biochemistry. After stepping down as director in 1999, Borst continued running his lab studying two topics: The biosynthesis and function of DNA base J, discovered by his group in trypanosomatids in 1993; and mechanisms of drug resistance in cancer cells.

Borst always spent most of his time doing research, but he also served on numerous scientific advisory boards and prize committees. He is a member of the Innovation Platform (a small think-tank chaired by the Dutch prime-minister), a foreign member of the British Royal Society and of the National Academy US.

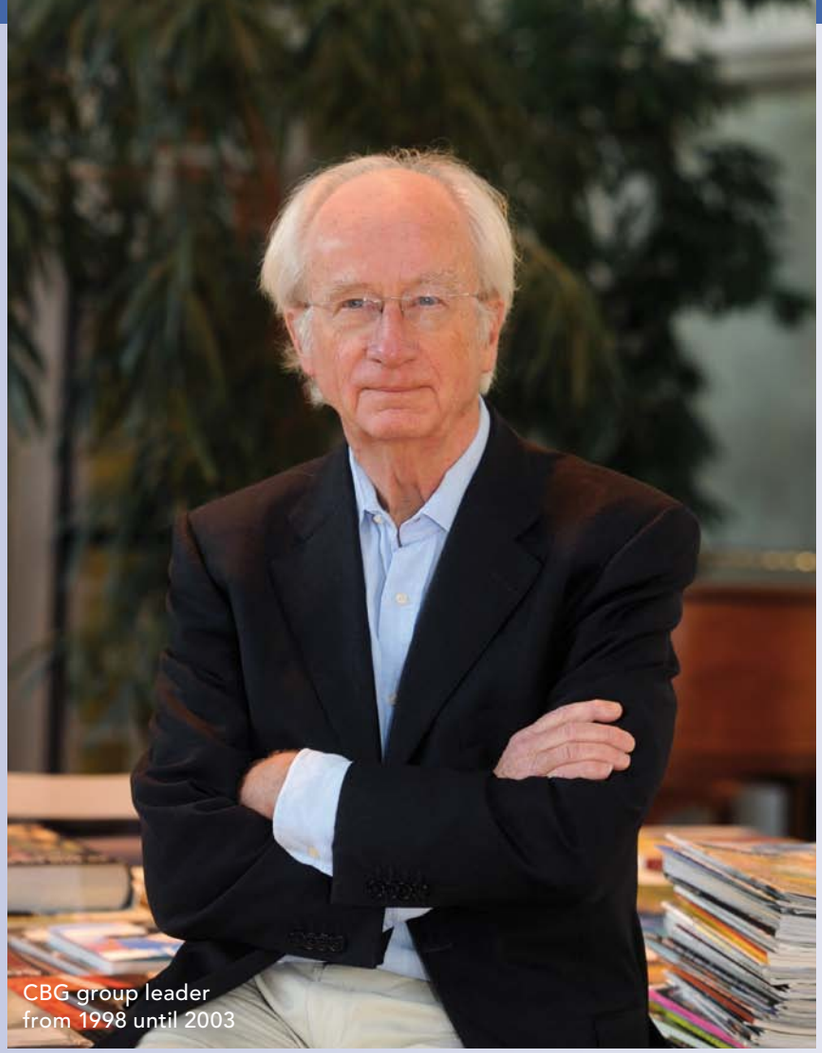
Trypanosoma – a soldier in the war on cancer

'A terrific encouragement.' That is how Piet Borst regarded the CBG grant received by the Netherlands Cancer Institute (NKI) and his research group. In 1998, when Borst became one of the first participants in the CBG collaboration, he was close to 65 years old, but he was far from finished with his scientific research. 'The CBG selection confirmed that I could still follow the scientific pace. It gave a powerful boost to my research and has been an incentive to continue', stated Borst, who is still group leader at the division of Molecular Biology at the NKI. He says also – not without pride – of the investigators within the CBG: 'It has also been a kind of family reunion of researchers who went "through my laboratory" in the past. Sometimes I was a father figure to them.'

Such comments might give the wrong impression – that the early CBG meetings were cosy family affairs, but in fact they were totally official scientific meetings. 'It was a most pleasant, non-bureaucratic assembly of people who had their roots in molecular biology and the same vision on how science should be done. The fun part was that CBG provided non-bureaucratic funding for a group of capable researchers who used it for highly interesting science. We have done a lot together and technology was the driving force behind our cooperation. And the participants have always been generous in sharing their technology with others.'

The significance of worm base 'J' to cancer in mammals

The research topics of Borst are peripheral to the



CBG group leader from 1998 until 2003

Goals

To elucidate the biosynthesis and function of base J, a new base that we discovered in the DNA of pathogenic protozoa, such as African trypanosomes; to study cancer chemotherapy in "spontaneous" tumours arising in genetically modified mice to determine mechanisms of drug resistance and to develop tools to detect and predict these.

Facilities and expertise

Expertise in molecular parasitology, in drug resistance mechanisms and cancer metabolism.

CBG: the study of the trypanosome parasite and investigation of the cellular mechanisms of drug resistance. According to Borst: 'We have done some research with the CBG-funded equipment, but the centre also gave me the opportunity to research topics that I couldn't have investigated otherwise.' In 1993 Borst's group discovered a unique base in the DNA of trypanosomes. Their DNA features a very small percentage of a base 'J', in addition to the usual bases A, C, T and G – about 1 percent of the base T has been replaced by the base J (ß-glucosyl-hydroxymethyluracil). Borst explains: 'Base J appears to be only present in the class of parasites that includes *Trypanosoma* and *Leishmania*. And base J is crucial for these parasites. If you replace all the J with T, *Leishmania* is not viable. We investigated the function and biosynthesis of base J and characterised the enzymes involved in the first step in its synthesis, namely the hydroxylation of base T. These enzymes are the J-binding proteins JBP1 and JBP2.'

'Finding tumour markers that predict the response to a drug is crucial.'

However, base J seemed to be an aberration, only of interest to basic researchers, offering little advantage to Borst's colleagues at the NKI. 'Silly Piet with his base J' is the mood recalled by Borst. That was 'until the day that a smart American bioinformation scientist found similar enzymes in humans and other mammals'. This family of TET proteins was found to be involved in the (de-)methylation of the base methyl C in mammalian DNA. Methylation of C is a so-called epigenetic marker, and it shuts down the activity of the genes in the methylated area of the DNA. Borst recounts: 'We had found something fundamental in those daft parasites – never seen in other organisms. Now it seems to have a connection with the regulation of genes in mammals! It seems the production of base J may now be important in the activation or de-activation of mammalian genes, and this process is crucial for understanding cancer.'

PIET BORST, THE NETHERLANDS CANCER INSTITUTE AMSTERDAM

Key publications

1 Cross M, Kieft R, Sabatini R, et al (1999). The modified base J is the target for a novel DNA-binding protein in kinetoplastid protozoans. EMBO J ;18:6573-81.

2 Chaves I, Rudenko G, et al (1999). Control of variant surface glycoprotein gene expression sites in Trypanosoma brucei. EMBO J ;18:4846-55.

3 Wijnholds J, De Lange ECM, et al (2000). Multidrug-resistance protein 1 protects the choroid plexus epithelium and contributes to the blood -cerebrospinal fluid barrier. J Clin Invest ;105:279-85.

4 Wijnholds J, Mol CAAM, et al (2000). Multidrug-resistance protein 5 is a multispecific organic anion transporter able to transport nucleoside analogs. Proc Natl Acad Sci U S A ;97:7476-81.

5 Reid G, Wielinga P, Zelcer N, et al (2003). The human multi-drug resistance protein MRP4 functions as a prostaglandin efflux transporter and is inhibited by nonsteroidal antiinflammatory drugs. Proc Natl Acad Sci U S A ;100:9244-9.

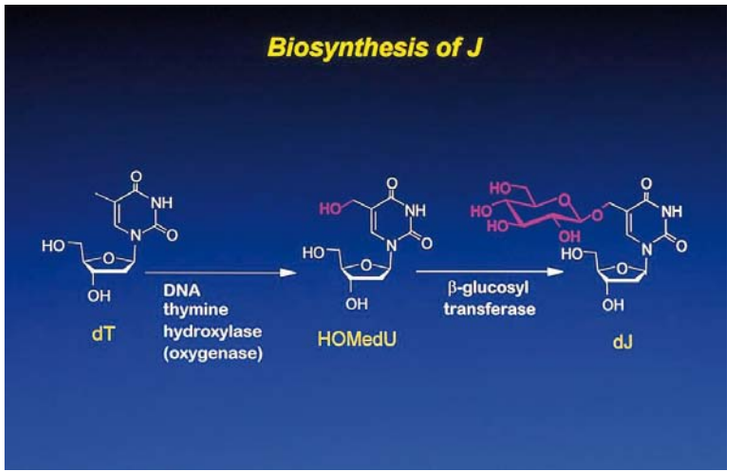
6 Zelcer N, Van de Wetering JK, et al (2005). Mice lacking multidrug resistance protein 3 show altered morphine pharmacokinetics and morphine-6-glucuronide antinociception. Proc Natl Acad Sci U S A ;102:7274-9.

7 Yu Z, Genest PA, ter Riet B, et al (2007). The protein that binds to DNA base J in trypanosomatids has features of a thymidine hydroxylase. Nucleic Acids Res ;35:2107-15.

8 Rottenberg S, Nygren AOH, et al (2007). Selective induction of chemotherapy resistance of mammary tumors in a conditional mouse model for hereditary breast cancer. Proc Natl Acad Sci U S A ;104:12117-22.

9 Rottenberg S, Kersbergen A, et al (2008). High sensitivity of BRCA1-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs. Proc Natl Acad Sci U S A ;105:17079-84.

10 Borst P, Rottenberg S, Jonkers J (2008). How do real tumors become resistant to cisplatin? Cell Cycle ;7:1353-9.



Biosynthesis of base J by modification of a specific thymine base in DNA.

From worm models to mouse models

The matter of drug resistance is another subject that has held Borst's attention for a long time. Why don't drugs do their job from day 1 in some patients? And why do other patients develop resistance during drug therapy? 'Local–regional cancer therapy, such as surgery and radiation, has become so excellent these days that people seldom die from the local consequences of a tumour any more.' Borst continues: 'Today's major problem is the metastasis of tumour cells. Patients often die in our hospital because there are no drugs left that can attack their tumour cells.' For many years Borst studied cellular pumps that jockey the drugs out of the cells, so the cells become insensitive to the drugs. The so-called P-glycoprotein pump is a notorious example. Among the places it can be found are the cells of the intestine and the blood–brain barrier, where it pumps anticancer drugs like paclitaxel out of the cells. 'We can inhibit those pumps very well with several compounds in tissue culture. We caused the pumps to slow down and we were convinced that we had found an important explanation for resistance to drugs. We also had good inhibitors to overcome that resistance', Borst recalls. 'But after that, studies in patients showed no consistent results. A huge discrepancy was shown between what we had found in the cells in the laboratory and what happens in patients. It was an immense disappointment.'

Group members

Staff
Henri van Luenen

Postdocs
Sven Rottenberg
Pankaj Tripathi
Koen van de Wetering

PhD students
Nikola Banishki
Petra Krumpochova
Guotai Xu MSc
Serge Zander

Technicians
Marcel de Haas
Liesbeth van Deemter
Sabrina Jan
Ariena Kersbergen
Sunny Sapthu
Wendy Sol

'We have done a lot together within the CBG and technology was the driving force behind our cooperation.'

Then a new type of mouse model was made at the NKI, using genetic modification. Two important tumour suppressor genes (*Brca1* and *p53*) were silenced in the breast tissue of those mice. It was quite different from the traditional nude mouse model with an immature immune system. Nude mice had been commonly used to study the effects of drugs in transplanted human tumours, but as Borst explained: 'Nude mice still have rudiments of their immune system that are able to clean up transplanted human tumour cells after a first hard blow due to the drug – an undesirable situation for studying the sole effect of a drug. The new mouse model provided us with a system in which tumours arose spontaneously, which could then be treated with anti-cancer drugs. These tumours respond more like human patients with cancer.'

Borst carried out experiments in these mice with the anti-tumour drug, docetaxel. He discovered that docetaxel switches on the P-glycoprotein pump – but at a much lower level than expected. Very low activity of this pump appears to be enough for the cells to create resistance to the drug, however this level cannot be detected by the methods now available in the clinic. Results from the mouse model have therefore shown the need for improved methods for detection of P-glycoprotein. Borst also uses the mouse tumour model to find biomarkers for predicting responses to a drug. 'In two out of every three patients treated with taxanes,' Borst says, 'the drugs have no effect at all on the tumour. But they do have adverse effects on the patients. So finding tumour markers that predict the response to a drug is crucial. I expect that our research will have a genuine impact in the clinic. We use new model systems to investigate questions we could not answer before, so we can better understand what happens in cells, better predict what will happen in tumours, and hence help oncologists eventually to treat cancer patients more effectively.'

HANS BOS, UNIVERSITY MEDICAL CENTER UTRECHT

CV

Hans Bos studied biology at the Free University, Amsterdam, and performed his PhD in the lab of Piet Borst, University of Amsterdam, working on yeast mitochondrial RNA. He received his PhD in1980. For his post-doctoral training he joined the group of Lex van der Eb at the University of Leiden to work on oncogenic transformation by adenovirus E1. In 1985 he started his work on the analysis of Ras mutations in human tumours and the function of Ras in oncogenic transformation. After a sabbatical year with Frank McCormick in San Francisco in 1990, he joined Utrecht University as professor in Physiological Chemistry, where he continued to work on small GTPases of the Ras family. Since 2007 he chairs the Division of Biomedical Genetics of the University Medical Center Utrecht.

Hans Bos is head of the Masters-PhD program Cancer, Genomics and Developmental Biology and director of the Cancer Genomics Centre of the Netherlands Genomics Initiative. He is a member of the Royal Netherlands Academy of Sciences and Arts.

Spatiotemporal dynamics of signal tr

Signal transduction systems enable cells to respond to changes in their environment. They are therefore of utmost importance to the wellbeing of each cell. Any dysfunction in the signalling system can lead to diseases like cancer. The research led by Hans Bos focuses on the molecular mechanisms and spatiotemporal dynamics of signal transduction networks mediated by Ras-like small guanosine triphosphate (GTP)-ases. One day this knowledge may have clinical applications.

Hans Bos is a member of the Centre for Biomedical Genetics as well as scientific director of the Cancer Genomics Centre – but he is not particularly interested in genes and DNA per se. As he says: 'Of course, DNA and genes are important, and cancer in particular is a disease of DNA. But personally I have always been more attracted to the *products* of DNA and genes – the proteins in the cell. In particular, those proteins that are involved in signal transduction. Signal transduction systems are – so to speak – the brain of the cell. The human brain senses all sorts of signals from inside and outside the body, and integrates these signals before deciding which action is most preferable at any particular moment. Similarly, signal transduction systems detect all relevant signals at the cellular level, integrate them and initiate suitable cellular activity. For example, a cell migrating on a surface receives signals from that surface and from the environment through which it migrates; these signals are integrated and trans-

CBG group leader since 1998

Goals

The aim of the lab is to understand the molecular mechanism of signal transduction in processes related to cancer. The focus is to understand molecular details of networks mediated by Ras-like small GTPases. Particular emphasis is on the spatial and temporal control of guanine nucleotide exchange factors, like the cAMP-regulated Epac protein, in cell adhesion, cell migration and metastasis.

Facilities and expertise

The expertise and facilities include biochemistry, molecular cell biology including confocal and live-imaging microscopy, structural biology and mass-spectrometry. Recent investments include Delta-vision live-imaging microscopes, LTQ-Orbitrap-Velos, Cellomics and peptide synthesizer.

ansduction networks

lated into movement in one particular direction. To perform that movement, the cell has to build up its cytoskeleton in one side of the cell at the same time as breaking the cytoskeleton down on the opposite side. The movement is only successful if all the biochemical processes involved in changing the cytoskeleton are tightly controlled in both time and space. This necessitates closely controlled spatiotemporal dynamics of several signal transduction systems in the cell.'

'The CBG provides an excellent breeding ground for future generations of scientists in the Netherlands.'

Ras and Rap1

The two signal transduction systems that Bos's group focuses on are the Ras and the Rap1 networks. Both are members of the family of so-called small GTPases. Bos explains: 'Small GTPases are membrane-bound molecular switches that are part of signalling networks involved in the control of cell proliferation, migration, adhesion and survival. In the early 1980s Ras was the first human oncogene to be discovered. Based on our earlier work, we now know that at least 15 percent of all tumours carry a mutation in the *Ras* gene.' Rather than analysing and deciphering other mutations in tumours, Bos is interested in finding answers to more fundamental questions: What is the function of the Ras protein in the cell and how is this function related to cancer? 'Our primary goal is to get an integrative picture of this signalling network and to understand its role in cellular processes such as metastasis. Finding a cure for a cancer patient would be a useful spin-off from that knowledge, but it is not our *primary* goal. It is important to bear in mind that the forms of

HANS BOS, UNIVERSITY MEDICAL CENTER UTRECHT

Key publications

1 K.A. Reedquist, E. Ross et al. The small GTPase Rap1 mediates CD31-induced integrin adhesion. J. Cell. Biol. 2000, 148:1151-1158.

2 J.M. Enserink, A.E. Christensen et al. A novel Epac-specific cAMP analogue demonstrates independent regulation of Rap1 and ERK. Nat. Cell Biol. 2002, 4:901-906.

3 S. Rangarajan, J.M. Enserink et al. Cyclic AMP induces integrin-mediated cell adhesion through Epac and Rap1 upon stimulation of the ß2-adrenergic receptor. J. Cell Biol. 2003, 160:487-493.

4 Price LS, Hajdo-Milasinovic A et al. Rap1 regulates E-cadherin-mediated cell-cell adhesion. J. Biol. Chem. 2004, 34:35127-35132.

5 Rehmann H, Das J, Knipscheer P, Wittinghofer A, Bos JL. Structure of the cyclic-AMP-responsive exchange factor. Epac2 in its auto-inhibited state. Nature, 2006, 439:625-628.

6 Rehmann H, Wittinghofer A, Bos JL. Capturing cyclic nucleotides in action: snapshots from crystallographic studies. Nature Rev. Mol. Cell Biol. 2007, 8: 63-73.

7 Bos JL, Rehmann H et al. GEFs and GAPs: critical elements in the control of small G proteins. Cell, 2007, 129:865-877.

8 Frische EW, Pellis-van Berkel W et al. RAP-1 and the RAL-1/exocyst pathway coordinate hypodermal cell organization in Caenorhabditis elegans. EMBO J. 2007, 26:5083-92.

9 Rehmann H, Arias-Palomo E et al. Structure of Epac2 in complex with a cyclic AMP analogue and RAP1B. Nature, 2008, 455:124-127.

10 Ponsioen B, Gloerich M et al. Direct spatial control of Epac1 by cyclic AMP. Mol. Cell. Biol. 2009, 29:2521-2531.

therapy for patients with cancer being introduced into the clinical arena now, are based on fundamental research that was performed 10 or 20 years ago. For example, drugs like imatinib and trastuzumab have come into existence thanks to previous research on signal transduction systems. Both drugs are targeted to signalling proteins connected to the Ras pathway.'

Rap1 leads to Epac

While studying the Ras signalling network, Bos's group stumbled on the Ras-like small GTPase, Rap1. 'Rap1 is the counterpart of Ras. Ras activation leads, for example, to a loosening of the contacts between adherent cells and it promotes cell migration, whereas activation of the Rap1 pathway strengthens cell to cell junctions and inhibits cell migration'. Therefore the group decided to shift gears and to work on this 'Ras-inhibitory pathway', digging further into the details of the Rap1 network. They deciphered many aspects of the network, but one particular highlight was the discovery of an activator of Rap1 – Epac – which turned out to be a novel cAMP target protein. This discovery changed the widely held belief that cAMP operates only through protein kinase A. 'This completely changed the cAMP field. In collaboration with other groups we developed an Epac-selective agonist.' This analogue has been used worldwide to identify more and more functions of Epac. Another highlight was when Holger Rehmann in Bos's group elucidated the crystal structure of Epac in its active and inactive forms. These structures explain precisely how Epac is activated upon binding of cAMP and how it activates Rap1. Furthermore, these structures are also being used to design further selective Epac agonists. Such drugs may have therapeutic value in processes that are regulated by Epac, such as regulation of endothelial barrier function in inflammation and in insulin secretion.

A fertile breeding ground

A large part of the research undertaken by Bos's group would have been impossible without the support of CBG. Bos emphasises: 'In the last ten to fifteen years, biomolecular research has changed tremendously. Techniques such as mass spectrometry, fluorescence microscopy, microarray and small

Current group members

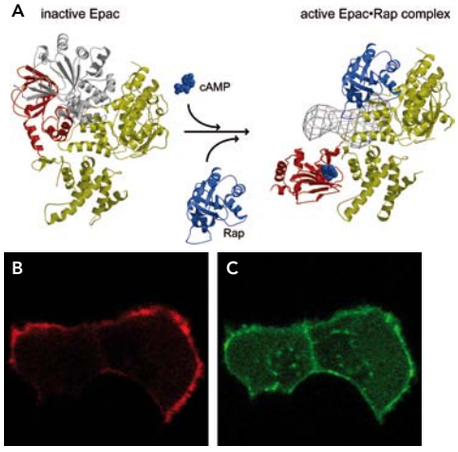
Staff
Fried Zwartkruis
Holger Rehmann

Postdoc
Sarah Ross

PhD students
Nayara Cavalcante Silva
On Ying Chan
John van Dam
Jantine van Dijk
Martijn Gloerich
Marlous Groenewoud
Lars Meijer
Willem-Jan Pannekoek
Milica Popovic
Anneke Post
Emma Spanjaard
Anouk van Veen

Technicians
Wendy van Berkel
Marije Rensen
Ingrid Verlaan
Marjolein Vliem

'Another important aspect of the CBG is the strong leadership of members in various areas of molecular biomedical research. This makes it possible to rapidly anticipate new developments.'



Structure of Epac2 in the inactive and active conformation.

Binding of cAMP induces a major change in the conformation that uncovers the catalytic helix for Rap1 binding (Rehmann et al., Nature, 2008, 455:124-127). Yellow, catalytic region; red, cAMP binding site, gray, DEP domain and second cAMP binding site, blue Rap1 and cAMP. The structure is determined by X-ray crystallography except for the gray sphere which was determined by single particle EM.

interfering RNA (siRNA) screening have been introduced. These techniques enable the study of signal transduction systems in more detail and – for the first time in history – they provide a comprehensive picture of their spatiotemporal dynamics. This is a very exciting area for researchers interested in the molecular mechanisms of signalling. However, these techniques are very expensive and demand expert knowledge. The CBG has invested heavily in developing these techniques and in building the necessary infrastructure. Some of the research in our laboratory has been performed in collaboration with other members of the CBG and, very importantly, the CBG has made these technologies available to many other groups so that even newly starting groups have profited from the CBG environment and been able to develop their talents. By their involvement, the CBG provides an excellent breeding ground for future generations of scientists in the Netherlands.'

Strong leadership

Bos adds: 'Another important aspect of the CBG is the strong leadership of members in various areas of molecular biomedical research. This makes it possible to rapidly anticipate new developments. As such, the CBG has been and will be a true leader of biomedical research in the Netherlands'. Indeed, among the members of the CBG are research directors of well-known organisations like the Netherlands Cancer Institute (NKI) and the Hubrecht Institute, directors of large research consortia in the Netherlands such as the Cancer Genomics Centre, the Centre for Medical Systems Biology and the Netherlands Proteomics Center.

BOUDEWIJN BURGERING, UNIVERSITY MEDICAL CENTER UTRECHT

CBG group leader
since 2004

Reactive oxygen species – the good,

By following the trace of one particular signal transduction system and continuously discovering more details about it, Boudewijn Burgering – often to his great surprise – came across various regulatory mechanisms involved in diseases like cancer and diabetes, as well as mechanisms behind the regulation of cell death and lifespan. His studies of reactive oxygen species (ROS) revealed that – depending on the cellular conditions – ROS can show a different face: namely, the good, the bad and the ugly.

'First and foremost I am interested in the way signalling within the cell and between cells takes place', explains Burgering about his research. 'I want to know how intracellular communication is regulated and how cells communicate between themselves. While studying these signal transduction pathways and steadily unravelling more details about them, you inevitably encounter aspects about transduction that also shed a new light on the cause of a certain disease, like cancer or diabetes, or even about a common process like aging. However, my research is not directly aimed at finding a cure for a particular disease. It aims primarily to improve our understanding of how cellular signal transduction systems work. Thereafter, this new knowledge may also have practical implications. You can never predict where such research will take you. This is why being a member of the CBG is important to me; their financial support makes it relatively easy for me to explore new areas, based upon our most

CV

Following graduation in Molecular Sciences (Wageningen University), Boudewijn Burgering started his PhD in 1986 at Leiden University studying the role of the small GTPase Ras in signal transduction. This was also the subject of study during his postdoc done in the lab of Hans Bos. This work resulted in some seminal findings within signalling regulated by Ras. In collaboration Paul Coffer, he identified the kinase PKB as acting downstream of the lipid kinase PI3K in insulin signaling. Further studies identified a plethora of substrates for PKB, most importantly Forkhead box O (FOXO) transcription factors. Interestingly, the pathway uncovered hereby, has by now been shown to play an important role in ageing and disease, e.g. cancer and diabetes. Consequently, PI3K/PKB signalling to FOXO serves now as the paradigm to study the relation between lifespan and disease onset. Burgering focusses on further unraveling molecular details and cellular consequences thereof. This resulted in a recent focus on Reactive Oxygen Species (ROS) regulation of and by FOXO. Again, ROS are believed to be central to ageing and disease and as such our studies provide rationale for this believe.

Goal

To understand at the molecular level the contribution of lifespan to disease onset employing the PI3K/PKB/FOXO pathway as the central paradigm

Facilities and expertise

How to apply state-of-the-art technology to uncover novel molecules/pathways in signal transduction. In house, CBG funded, facility was a LC-MS/MS but this is recently replaced by VELOS-Orbitrap.

the bad and the ugly

recent research findings. The CBG can also be confident that each member receiving a grant will spend it in a sensible way, and this confidence is based on the proven high quality of those members. Compared to other organisations, the CBG is less demanding about the data it requires to explain why I want to explore a particular line of research. This means the CBG gives me more freedom to choose which direction our research takes, or to choose an entirely new direction.'

'Following the trace of one particular signal transduction pathway led us to discover more about two age-related diseases (cancer and diabetes), as well aging itself.'

Cancer, diabetes and lifespan

Thanks to that degree of freedom, Burgering has been able to follow several lines of research that emerged from his original topic – the protein kinase B (PKB). 'Together with Paul Coffer I discovered the serine–threonine protein kinase PKB as a target for PI-3K signalling. Activation of PI-3K, the lipid kinase phosphoinositide 3-kinase, results in the formation of 3' phosphorylated inositol lipids. These lipids have long been suspected to function as second messengers, and PI-3K activity has been shown to be essential to many cellular processes, including cell cycle control, cell survival and cell metabolism. By further exploring the role of protein kinase B, we and other researchers were able to show that mutations in this signal transduction pathway are involved in cancer as well as in diabetes. In the former, these mutations lead to activation of PI-3K/PKB while in the latter they lead to inactivation of the pathway. Additionally we showed that PI-3K/PKB activation is involved in the regulation of Forkhead box O transcription factors (or

BOUDEWIJN BURGERING, UNIVERSITY MEDICAL CENTER UTRECHT

Key publications

1 Kops GJ, de Ruiter ND et al (1999). Direct control of the Forkhead transcription factor AFX by protein kinase B. Nature ;398(6728):630-4.

2 Medema RH, Kops GJ, Bos JL, Burgering BM (2000). AFX-like Forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through p27kip1. Nature.;404(6779):782-7.

3 Brownawell AM, Kops GJ, Macara IG, Burgering BM (2001). Inhibition of nuclear import by protein kinase B (Akt) regulates the subcellular distribution and activity of the forkhead transcription factor AFX. Mol Cell Biol.;21(10):3534-46.

4 Kops GJ, Medema RH et al (2002). Control of cell cycle exit and entry by protein kinase B-regulated forkhead transcription factors. Mol Cell Biol.; 22(7):2025-36.

5 Kops GJ, Dansen TB et al (2002). Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. Nature. Sep 19; 419(6904):316-21.

6 Van der Horst A, Tertoolen LG et al (2004). FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2(SIRT1). J Biol Chem. ;279(28):28873-9. Epub 2004 May 4.

7 Ral and JNK. Essers MA, Weijzen S et al (2004). FOXO transcription factor activation by oxidative stress mediated by the small GTPase. EMBO J. ;23(24):4802-12. Epub 2004 Nov 11.

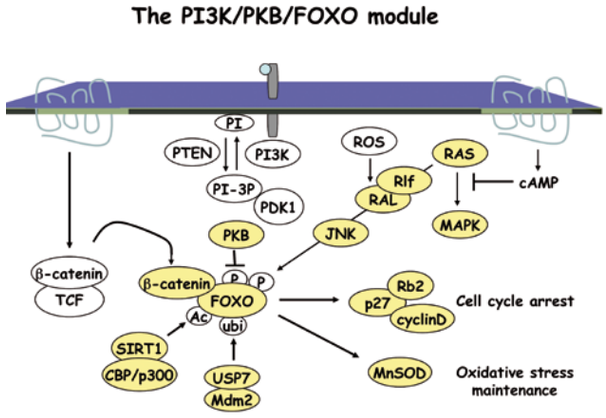
8 Essers MA, de Vries-Smits LM et al (2005). Functional interaction between beta-catenin and FOXO in oxidative stress signaling. Science. 20;308(5725):1181-4.

9 Van der Horst A, de Vries-Smits AM et al (2006). FOXO4 transcriptional activity is regulated by monoubiquitination and USP7/HAUSP. Nat Cell Biol.;8(10):1064-73. Epub 2006 Sep 10.

10 Dansen TB, Smits LM et al (2009). Redox-sensitive cysteines bridge p300/CBP-mediated acetylation and FoxO4 activity. Nat Chem Biol.;5(9):664-72. Epub 2009 Aug 2.

Signal transduction studied by the Burgering Lab.

Cartoon impression of signal transduction pathways regulated by the Ras and PI3K pathway. Those players identified amongst others by our lab are shaded in yellow.



FOXOs). This finding opened up a whole new area of research to us on aging and lifespan. FOXOs are not only involved in processes like cell proliferation and insulin signalling, but they are also known to be involved in cell death through apoptosis. This shows how following the trace of one particular signal transduction pathway led us to discover more about two age-related diseases (cancer and diabetes), as well aging itself.'

Not simply 'evil'

In recent years the Burgering group has further explored the details of FOXO regulation. 'By doing so we came upon yet another interesting – and also highly controversial – topic. Reactive oxygen species (ROS) are mostly thought of as 'evil' because they are involved in the etiology of a number of diseases of the elderly such as cancer, diabetes, hypertension, chronic kidney diseases and atherosclerosis. This led to the general assumption that the use of antioxidants to lower the concentration of ROS in the body is beneficial. However, by further elucidating the signal transduction pathways that ROS are involved in, we gradually became aware that ROS are not simply 'evil'. In fact, ROS have a role in several interconnected signal transduction pathways, and in normal conditions they are even beneficial to the cell. Only when concentrations of ROS increase above a certain level do they become 'evil'.

In a recent paper we described these different faces of ROS as 'the good, the bad and the ugly'. If they are present in low concentrations (which is the case in normal physiological conditions) they

Current group members

Staff
Tobias Dansen

Postdoc
HarmJan Vos

PhD students
Diana Hoogeboom
Astrid Eijkeleboom
Marrit Putker
Anna Szypowska
David Kloet
Maaike vd Berg
Pareskevi Charitoe

Technicians
Lydia Smits
Miranda van Triest
Hesther de Ruiter
Iris van Zutphen
Pauline Riedl-Polderman

'The CBG gives me more freedom to choose which direction our research takes, or to choose an entirely new direction.'

are 'good' and act as second messengers in the regulation of transcription factors such as FOXO, HIF-1 and TCF (hypoxia-inducible factor 1 and T-cell factor, respectively). However, a sudden, strong increase in ROS reveals their 'ugly' face; the increase triggers a robust anti-ROS defence response, which induces senescence or cell death. A moderate increase in ROS is not capable of triggering this strong defence response, and this is when the ROS show their 'bad' face, whereby they cause cellular damage, but without inducing cell death. It is this 'bad' face that can cause diseases like cancer or diabetes.'

Still a lot of blank spaces

By constantly digging deeper and deeper into these signal transduction systems, Burgering became (sometimes quite surprisingly) embroiled in different health issues. 'Although in recent years we have made a lot of progress in our understanding of these signal transduction pathways and their involvement in disease and aging, the picture is not yet complete. There are still a lot of blank spaces in our models. This becomes obvious every time an attempt is made to translate this fundamental knowledge to the clinic. For example, our research upon the Ras pathway, which comes into play in several forms of cancer, led to the development of agents that are able to inhibit this pathway. Indeed, recent evidence from clinical trials shows that agents that inhibit downstream Ras signalling are capable of shrinking tumours. However, in extreme cases these agents simultaneously cause the development of other sorts of tumours. The same is true of antioxidants. It has been shown that ROS are involved in the development of tumours, yet the use of antioxidants does not protect people against cancer. In fact, a recent meta-analysis showed that regular consumption of some types of antioxidants may even increase the chance of getting cancer. Obviously, our models do not yet describe the real world, but this does not mean that this kind of fundamental research is useless. It simply means that we have to keep on delving further into the details of these pathways – because the devil is always in the detail! For me it is like trying to solve the biggest Sudoku puzzle imaginable. And working towards that goal, together with other members of the CBG, is very rewarding!'

HANS CLEVERS, HUBRECHT INSTITUTE UTRECHT

CBG group leader
since 1998

Identifying (cancer) stem cells

Stem cells are the starting material for the formation of every organism and every living tissue. To understand at a molecular level how stem cells are stimulated to divide, proliferate and differentiate is of great interest to scientists other than developmental biologists. This knowledge will guide us on how to use stem cells in regenerative medicine and how to intervene in diseases like cancer. Studying a particular signal transduction pathway that later proved to be an important activator of stem cells, Hans Clevers became an internationally acknowledged pioneer in the area of stem cell research, as well as in cancer research.

Hans Clevers is the director of the Hubrecht Institute for Developmental Biology and Stem Cell Research, so one would certainly expect him to be busy doing research on embryos; however, that is not all he does. Most of the pioneering data obtained in recent years came from his studies of the small intestine in mice. 'For a scientist interested in developmental biology, the small intestine is a very useful tissue', Clevers explains. 'The intestinal epithelium is regenerated throughout adult life. In the mouse, for example, the whole epithelium is renewed every five days. This process is fuelled by stem cells that reside near the bottom of the crypts in the intestinal epithelium. So the intestinal epithelium, especially those crypts, is an excellent model to study stem cells and the molecular and genetic processes involved in the development of new, differentiated tissues from stem cells.'

CV

Hans Clevers obtained his MD degree in 1984 and his PhD degree in 1985 from the University Utrecht, the Netherlands. His postdoctoral work (1986-1989) was done with Cox Terhorst at the Dana-Farber Cancer Institute of the Harvard University, Boston, USA.

From 1991-2002 Hans Clevers was Professor in Immunology at the University Utrecht and, since 2002, Professor in Molecular Genetics. Since 2002, he is Director of the Hubrecht Institute in Utrecht.

Hans Clevers has been a member of the Royal Netherlands Academy of Arts and Sciences since 2000 and is the recipient of several awards, including the Dutch *Spinoza Award* in 2001, the Swiss *Louis Jeantet Prize* in 2004, the Memorial Sloan-Kettering *Katharine Berkan Judd Award* in 2005, the Israeli *Rabbi Shai Shacknai* Memorial Prize in 2006, and the Dutch *Josephine Nefkens Prize for Cancer Research* and the German *Meyenburg Cancer Research Award* in 2008. He obtained an ERC Advanced Investigator grant in 2008. He is Chevalier de la Legion d'Honneur since 2005.

Goals

To understand the molecular mechanisms of Wnt-driven self-renewal and carcinogenesis and uncover the commonalities between the two processes. To identify the elusive intestinal stem cell and the determination of its role in self-renewal and carcinogenesis

Facilities and expertise

A histopathology unit and a 2-photon confocal laser microscope for phenotyping of mouse and human tissues; an Agilent micro-arrayer and a MoFlo cell sorter for gene expression profiling on sorted (stem and cancer) cells. Unrestricted access to the Hubrecht transgenesis/knockout mouse and zebrafish facility, as well as the Hubrecht deep-sequencing facility. An active collaboration with the Netherlands Proteomics Facility of Albert Heck

A marker for identifying stem cells

One disadvantage when working with stem cells in whole (adult) tissue is that first of all you have to be able to identify the stem cells correctly. Clevers has this to say: 'Recently we developed a method to accurately identify stem cells in the intestinal epithelium in the mouse. The method is based on our knowledge of the signal transduction systems involved in stem cell activation. More than decade ago, we showed that the Wnt signalling pathway plays an important (perhaps universal) role in the activation of stem cells. Abrogation of this pathway – by removal of Tcf4 or beta-catenin (transcription factors that both are part of the Wnt signalling pathway) or by over-expression Dkk-1 (an inhibitor of Wnt) – results in a complete failure of proliferation of the stem cells. Then around seven years ago, we showed that the activated Wnt pathway in colon cancer cells induces the transcription of about 80 different target genes. A closer look at these genes reveals that expression of the gene *Lgr5* is a good marker for identifying stem cells in the intestinal epithelium. Expression of *Lgr5* proved to be restricted to rare, scattered cells in the bottom of the crypts. To visualise the expression of *Lgr5* we made a mouse in which we knocked in a cassette encoding for green fluorescent protein in the *Lgr5* gene together with a tamoxifen-inducible Cre-recombinase enzyme. This allowed us to mark cells in the intestinal crypts expressing *Lgr5* and their progeny. By doing so we were able to prove that the cells that rapidly renew the intestinal epithelium all arise from a very limited number of stem cells located in a distinct area at the bottom of the epithelial crypts. This finding also suggests that these stem cells are dividing constantly and rapidly throughout adult life. This is in sharp contrast with "Hayflick's limit" which states that stem cells only divide a finite number of times.'

'It is obvious that the signalling pathways we study within the scope of developmental biology are potentially involved in the aetiology of cancer.'

HANS CLEVERS, HUBRECHT INSTITUTE UTRECHT

Key publications

1 Van de Wetering, M., Sanchoet al (2002). The beta-catenin/ TCF4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. Cell 111: 241-250.

2 Battle, E., Henderson, J.T. et al (2002). Beta- catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/ephrinB. Cell 111: 251-263.

3 Haramis A.P., Begthel H. et al (2004). De novo crypt formation and Juvenile Polyposis upon BMP inhibition Science. 303:1684-6.

4 Van Es J.H., Van Gijn M.E. et al (2005). Notch pathway/ gamma-secretase inhibition turns proliferative cells in intestinal crypts and neoplasia into Goblet cells. Nature 435:959-963.

5 Batlle E., Bacani J. et al (2005). EphB activity suppresses colorectal cancer progression. Nature 435:1126-1130.

6 Clevers, H. (2006). Wnt/ß-catenin signaling in development and disease. Cell 127: 469-480.

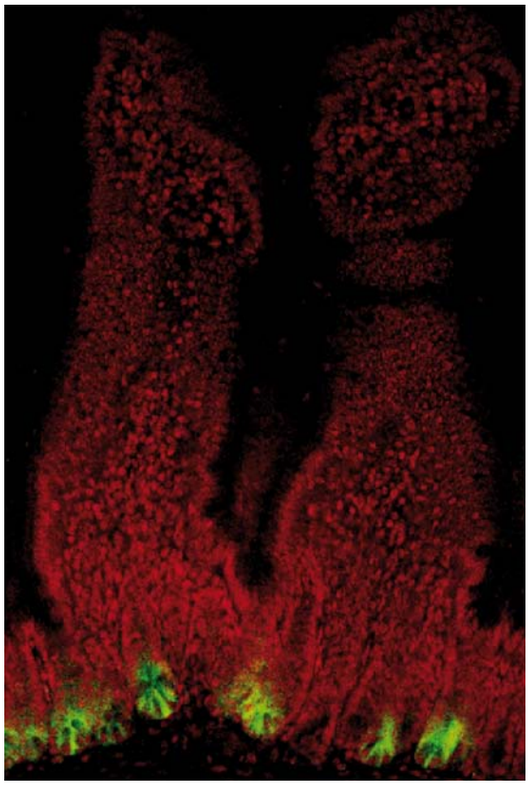
7 Barker, N, van Es et al (2007). Identification of Stem Cells in Small Intestine and Colon by a Marker Gene LGR5. Nature, 449:1003-1007.

8 Barker N., Ridgway R.A. et al (2009). Crypt Stem Cells as the Cells-of-Origin of Intestinal Cancer Nature 457:608-611.

9 van der Flier, L.G., van Gijn et al (2009). Transcription factor Achaete scute-like 2 (Ascl2) controls intestinal stem cell fate. Cell 136: 903-12.

10 Sato, T., Vries, R. et al (2009). Single lgr5 gut stem cells build crypt-villus structures in vitro without a stromal niche. Nature 459 :262-5.

The epithelium of the intestine consists of large protrusions which carry the differentiated cells that mediate digestion and uptake of food. These cells only live for about 3 days. As a consequence, the tissue has to be renewed at very high rate. Crypts, small invaginations at the base of the crypts contain stem cells and rapidly dividing undifferentiated daughter cells that are produced by the stem cells. In the image the nuclei of all cells are stained with red. Two large villi point upwards. Hidden at their base are about ten crypts. By inserting Green Fluorescent Protein into a stem cell gene discovered in our lab, Lgr5, we revealed the presence of small (green) stem cells at the bottom of the crypts (Barker et al, Nature 2007)



The existence of (cancer) stem cells

Although unravelling the molecular pathways that are involved in activation and differentiation of stem cells is the main aim of Clevers' group, inevitably this line of research also feeds into health issues – and cancer in particular. 'Cancer is, as we now know, the result of defects in the pathways that control the division, proliferation and differentiation of cells. It is obvious that the signalling pathways we study within the scope of developmental biology are potentially involved in the aetiology of cancer. Indeed, in recent years we have shown that the Wnt signalling pathway, if activated irreversibly for example by mutational loss of the tumour suppressor gene *Apc* can lead to adenomatous transformation of the intestinal epithelium and eventually colon cancer. This opens new avenues for therapeutic manipulation of the Wnt pathway in the treatment of colon cancer. The same is true for another recent observation in our laboratory: inhibition of the so-called Notch signalling pathway induces stem cells in the intestinal epithelium to differentiate into goblet cells. The Notch signalling pathway can be blocked by molecules that inhibit the

Current group members

Postdocs
Rik Korswagen
Robert Vries
Nick Barker
Adam Hurlstone
Daniel Pinto

PhD students
Annette Baas
Laurens van der Flier
Daniel Stange

'Recently the theory has been proposed that cancer growth is fuelled by only a small number of stem cells. If proven to be right, this could change the way we look at treating cancer – cancer stem cells would be our main target.'

activity of gamma secretase. Therefore we set up a small company to investigate the use of these gamma-secretase inhibitors in a therapeutic setting.'

In addition, the method devised by Clevers' group to identify stem cells *in vivo* may also provide an answer to the most burning issue in cancer research at this moment, namely: Do cancer stem cells really exist? Clevers says: 'Recently the theory has been proposed that cancer growth is fuelled by only a small number of stem cells. If proven to be right, this could change the way we look at treating cancer – cancer stem cells would be our main target. However, until now it has been difficult to support this theory and to demonstrate the existence of these putative cancer stem cells. By interbreeding the mice we used to identify intestinal stem cells with *Apc* mice (which are prone to develop intestinal adenomas) we were able to show that activation of the *Lgr5* gene only takes place in a limited number of cells within large adenomas. It is very tempting to label these cells as cancer stem cells. Research is going on to further support this claim.'

No need to declare milestones

In order to perform the kind of pioneering research described above, it is essential to be flexible and to be able to rapidly implement new strategies. 'That is one of the advantages of being a member of the CBG', Clevers states. 'Grants from the CBG are given without needing to give precisely defined milestones. The nature of the research we perform in the lab makes it very difficult to describe any milestones in advance. You never know where the data will take you, which is why the CBG grants are very important to us, especially as they also allow us to perform so-called 'risky research'. Finally, the CBG provides a good and accessible environment for the exchange of new scientific knowledge and techniques. So, for example, when we started to use microassays, we first visited our colleagues in Amsterdam who were far more experienced at the technique, and now we are showing other members of the CBG our technique for identifying stem cells *in vivo*.'

PETER TEN DIJKE, LEIDEN UNIVERSITY MEDICAL CENTER

CV

Peter ten Dijke received his PhD degree in 1991 from Wageningen University, The Netherlands based on his research on the identification of the third isoform of TGF-ß performed at Oncogene Science, Inc., New York, USA. He did his postgraduate studies with Kohei Miyazono and Carl-Henrik Heldin at the Ludwig Institute for Cancer Research (LICR), Uppsala, Sweden. In 1994, he became group leader at LICR and in 1999 he moved to the Netherlands Cancer Institute, Amsterdam, The Netherlands. In 2005 he moved to the Leiden University Medical Center (LUMC), Leiden, The Netherlands, and is currently a professor of molecular cell biology at Leiden University. His laboratory studies the molecular mechanisms by which TGF-ß family members elicit their cellular effects via (co) receptors and intracellular SMAD effectors, and how subverted TGF family signaling is involved in cancer, vascular and bone diseases.

Goals

To identify novel critical regulators of TGF-ß and Wnt family signaling pathways and to analyze their mechanism of action. To identify key regulators of tumour angiogenesis, and to explore their potential as targets for anti-angiogenesis therapy. To demonstrate that two diseases with too much bone formation, i.e. fibrodysplasia ossificance progressiva and sclerosteosis, are caused by overactive BMP and Wnt growth factor signaling.

The critical component in a signalling pathway

On the wall in his room at the Leiden University Medical Center (LUMC) science building, Peter ten Dijke has put up a poster charting the known signalling pathways in human cancer. Hundreds of biologically active molecules are arranged in a flow-chart that dazzles the eye. To interfere with such a complex system in order to cure a disease seems almost impossible. And that is precisely what he is good at – knowing where to begin. Or as he himself puts it: 'Our expertise is finding the critical component in a complex signalling pathway.'

Ten Dijke is relatively new to the CBG 'super-family'. He joined in 2009. 'Over the last few years, we have been focused on translational research, trying to bring some of our results closer to clinical application. It was a fascinating challenge, but it also became clear that it is quite another ball game. Now that companies are coordinating the clinical trials on those targets, it is time to go back to our core business: defining novel key elements in signal transduction. So that is what we are doing again. Thanks to the CBG, we have several new people in our team who are eager to explore new paths.'
'CBG funding has also made it possible for us to participate in the purchase and design of a unique microscope: it combines electron microscopy and light fluorescence microscopy. This combination allows us to examine specimens with both kinds of microscopy in one and the same set-up. We can

CBG group leader since 2009

Facilities and expertise

Technology platforms for genomics, proteomics and bioinformatics. Transcriptional profiling and analysis of interacting proteins by mass spectroscopy. The use of molecular imaging in mice to study tumour progression and metastasis, angiogenesis, bone formation and alterations in specific signalling pathways. Our department provides a core facility for light and electron microscopes, including confocal and fluorescence microscopy, life cell imaging, intravital microscopy and transmission electron miscroscope. In 2009 with support of CBG a light electron microscope (LEM) was designed and purchased. This will allow us to combine light microscope with electron microscopic analysis, in a correlative way: CLEM (Correlative Light and Electron Microscopy).

highlight an area of interest with a fluorescent tag and then zoom in with electron microscopy to levels of magnification far beyond the limitations of light microscopy.'

Dual role in cancer

Transforming growth factor-beta (TGF-ß) has been the focus of Ten Dijke's research since the late 1980s, when he discovered the third isoform of this cytokine. His lab was among the first to identify and characterise the role of transmembrane serine/threonine kinase receptors and Smad transcription factors that carry the TGF-ß signal from the plasma membrane to the nucleus of the cell. 'The different TGF-ß pathways play critical roles in both embryonic development and adult tissue homeostasis. We study the involvement of the TGF-ß/Smad pathways and their regulatory molecules in the metastasis of cancer cells, vascular disorders and high/low bone mass diseases.' The effects of TGF-ß signalling can be paradoxical. TGF-ß inhibits unwanted cell growth. To continue to proliferate, early cancer cells have to turn a deaf ear to TGF-ß-induced growth inhibitory signals. But at a later stage, other TGF-ß signals actually stimulate many cancer cells to de-differentiate (epithelial to mesenchymal transition, EMT) and metastasise.

Transforming growth factor-beta (TGF-ß) has been the focus of Ten Dijke's research since the late 1980s, when he discovered the third isoform of this cytokine.

The Leiden team studied the signals involved in EMT and metastasis in breast tumour cells. They found that Smad4 – initially identified as tumour suppressor in pancreatic cancer – was required for TGF-ß-induced EMT and bone metastasis of breast cancer cells. 'We subsequently investigated the role of the Smad4-binding proteins, Smad2 and Smad3. Knock-down of Smad3 in breast cancer cells resulted in prolonged latency and delayed growth of bone metastasis. But intriguingly, Smad2 depletion resulted in more aggressive metastatic tumours by promoting tumour angiogenesis, the formation of new blood vessels.'

PETER TEN DIJKE, LEIDEN UNIVERSITY MEDICAL CENTER

Key publications

1 Itoh F, Asao H, et al (2001). Promoting bone morphogenetic protein signaling through negative regulation of inhibitory Smads. EMBO J 20,4132-42.

2 Goumans M-J, Valdimarsdottir G, et al (2002). Balancing the activation state of the endothelium via two distinct TGF-ß type I receptors. EMBO J 21,1743-53.

3 Korchynskyi O, ten Dijke P. (2002) Identification and functional characterization of distinct critically important bone morphogenetic protein-specific response elements in the Id1 promoter. J Biol Chem 277,4883-91.

4 Goumans M-J, Valdimarsdottir G, et al (2003). Activin receptor-like kinase (ALK)1 is an antagonistic mediator of lateral TGFbeta/ALK5 signaling. Mol Cell 12,817-28.

5 Lebrin F, Goumans M-J, et al (2004). Endoglin promotes endothelial cell proliferation and TGF-ß/ALK1 signal transduction. EMBO J 23:4018-28.

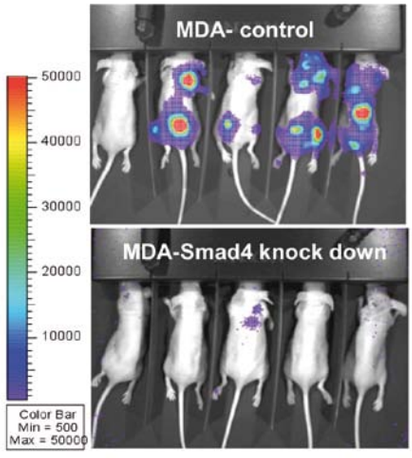
6 Deckers M, van Dinther M, et al (2006). The tumor suppressor Smad4 is required for transforming growth factor b-induced epithelial to mesenchymal transition and bone metastasis of breast cancer cells. Cancer Res 66:2202-9.

7 Scharpfenecker M, van Dinther M, et al (2007). BMP-9 signals via ALK1 and inhibits bFGF-induced endothelial cell proliferation and VEGF-stimulated angiogenesis. J Cell Sci. 2007;120:964-72.

8 ten Dijke P, Arthur HM. (2007) Extracellular control of TGFbeta signalling in vascular development and disease. Nat Rev Mol Cell Biol. 8:857-69.

9 Smits AM, van den Hengel LG, et al (2009). A new in vitro model for stem cell differentiation and interaction. Stem Cell Res. 2:108-12.

10 Liu, Z Kobayashi, K van Dinther, et al (2009). VEGF and TGF-beta type I receptor kinase inhibitor synergistically promote blood vessel formation by inducing integrin alpha5 expression. J. Cell Sci., 122:3294-302.



The knockdown of Smad4 in MDA-MB-231 breast cancer cells strongly inhibits the frequency of bone metastasis in nude mice. Control MDA-MB-231 cells (top panel) or MDA-MB-231cells (bottom panel) both expressing firefly luciferase, were intracardially injected in 5-week-old female nude mice and analysed after 35 days by biololuminescent imaging for metastasis. The number of bone metastasis of MDA-MB-231 cells are greatly reduced upon Smad4 depletion.

Angiogenesis switch

In tumour angiogenesis TGF-ß pathways also play a dual role. They can both stimulate and inhibit the formation of new blood vessels. This dual control makes it difficult to inhibit TGF-ß's stimulatory effect on cancer progression. To simply inhibit TGF-ß may help to stop metastasis and tumour vascularisation, but it might also encourage development of new tumours and have other unwanted side-effects. Luckily, most of these effects are mediated via different signalling pathways. For instance, Ten Dijke and his team demonstrated that during angiogenesis TGF-ß can activate two distinct type-I TGF-ß receptors in endothelial cells (ALK1 and ALK). TGF-ß–induced ALK1 signalling enhances angiogenesis by stimulating endothelial cell proliferation, migration, and tube formation, but TGF-ß-induced ALK5 inhibits these processes. 'We also found that the accessory receptor endoglin, an important component of TGF-ß receptor complexes, is required for ALK1 signalling, but can inhibit ALK5 signalling. This provides the endothelial cell with an intricately regulated control switch that offers possibilities for medical intervention. Together with several pharmaceutical companies we studied the effect of neutralising antibodies against ALK1 and endoglin. We also investigated the mechanism of action of a ligand trap for ALK1. All these compounds are currently being studied as potential angiogenesis-inhibiting drugs in cancer patients. Angiogenesis inhibition has yet to fulfil its promise in oncology. I believe that we may still get it to

Current group members

Staff
Hans van Dam
Marie-Jose Goumans

Postdocs
Erik Meulmeester
Anke Smits
Guy Roukens
FangFang Zhou
Long Zhang
Juan Zhang
Lucas Hawinkels
Evangelia Pardali
David de Gorter
Olexandr Korchynskyi
Eliza Wiercinska
Theo van Laar

PhD students
Hillegonda Naber
Carola Krause
Xiaofei Zhang
Angelique van Oorschot
SongTing Shi
Dwi Kemaladewi

Technicians
Midory Thorikay
Maarten van Dinther

work, if only we had several compounds acting on different pathways to circumvent resistance.'

Endoglin is not only part of the TGF-ß receptor complex, but it is also present in the circulation. High levels of soluble endoglin contribute to the pathogenesis of pre-eclampsia. Ten Dijke explains: 'We have investigated the mechanism by which endoglin is shedded from cells. We identified a specific matrix metalloprotease as the major endoglin-shedding protease. In addition, we found that soluble endoglin has strong inhibitory effects on angiogenesis.'

Bone and muscle

Many members of the TGF-ß superfamily function as growth and differentiation factors in the body. This offers perspectives for application in regenerative medicine. For instance, the bone morphogenetic proteins (BMPs) are powerful stimuli for bone and cartilage formation. Bone diseases such as osteoporosis have been studied by other groups in Leiden for decades. Ten Dijke elaborates: 'To identify critical targets involved in bone formation we have looked at a few rare diseases characterised by too much bone. We found that the BMP type-I receptor R206H point mutation involved in fibrodysplasia ossificans progressiva makes the BMP receptor complex constitutively active and sensitises mesenchymal cells to BMP-induced osteoblast differentiation and bone formation. Another disease Ten Dijke's group investigated was sclerosteosis, a skeletal disorder characterised by high bone mass, and caused by loss of the DAN family member sclerostin. We found that sclerostin is expressed by osteocytes and inhibits osteoblast differentiation by inhibiting the Wnt co-receptor LRP5 and LRP6 function.'

In other types of tissues, such as muscle, TGF-ß and related molecules are mainly known as growth inhibitors. 'You know those Belgian blue cows? They have a mutation in the gene for myostatin, a TGF-ß superfamily member that inhibits muscle growth. We are trying to selectively inactivate myostatin and TGF-ß in muscle, to restore the muscle power of patients with Duchenne's muscular dystrophy that are treated with the exon-skipping method of GertJan van Ommen and his group.'

LEX VAN DER EB, LEIDEN UNIVERSITY MEDICAL CENTER

CV

Lex van der Eb studied biology at the University of Leiden. His PhD research on the properties of Adenovirus DNA was carried out in Leiden (degree in 1968). After a postdoctoral period in professor Jerome Vinograd's laboratory at Caltech, Pasadena USA (1968-1969), he started a research group at the Medical Faculty of the University of Leiden. His work was focused on the mechanism of oncogenic transformation of cells by adenoviruses, functions of tumor suppressor genes and Adenovirus vector-mediated gene therapy. In 1980 he was appointed full professor in Tumor Virology. Around 1987 he began collaboration with prof. Dirk Bootsma's group on the isolation and characterization of human DNA repair genes. This collaboration was further extended, and resulted in the founding of the "Medisch Genetisch Centrum Zuid-West Nederland" (MGC).

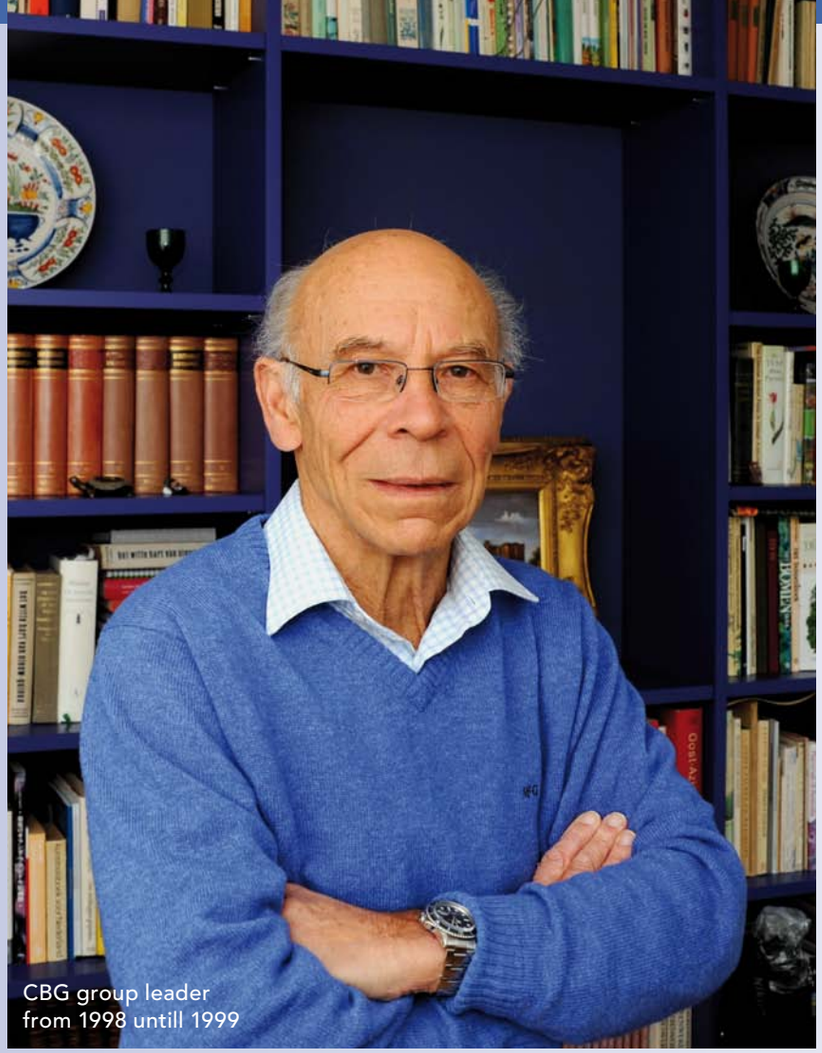
Van der Eb is a member of the Royal Netherlands Academy of Sciences and of EMBO. He has served in several Heineken Prize selection committee's and was a member of the Science Committee for the Louis-Jeantet Prize for medicine. He received several awards including the AKZO Prize and the Robert Koch prize. Van der Eb is presently senior advisor at Crucell, The Netherlands.

Viral proteins as tools to investigate

Lex van der Eb is one of the founding fathers of the CBG. His groundbreaking basic research on the transformation of cells and oncogenesis had been a strong foundation for diverse lines of scientific enquiry – from the properties of viral-transforming proteins and the p53 gene to the study of gene therapy. His contribution to the CBG was limited because he retired soon after it was established, but he still had much to say about his research and developments over recent years.

'The regulation of cell growth and the cell cycle is very complex – almost too complex to study. This was especially so when I started out in this field in the mid-1960s. That's why we chose to focus on the virus-induced transformation of cells. This way you know what external factors have been introduced, so you can study the way the molecules of the cell itself react to them. The approach became even more practical when Frank Graham in my lab devised a method back in 1973 for introducing DNA directly into the cell in such a way that it was biologically active. Thus we could single out individual parts of the viral genome and see how they influenced cell growth. This tool enabled us to identify the major proteins of the cell involved in regulation of the cell cycle.'

Interestingly, the type of virus – an adenovirus – that Van der Eb used through these decades of cancer research does not actually cause cancer in human cells. The worst disease humans can get



CBG group leader
from 1998 untill 1999

Goals

To unravel the mechanism of action of the E1A protein in cell transformation and oncogenity. To understand the functions of tumour suppressor proteins and of the ERCC3 DNA repair protein. To use adenovirus vectors in gene therapy.

Facilities and expertise

The laboratory of the group was part of the department of Molecular Cellbiology, Sylvius Laboratorium, Leiden. The expertise was focused on the molecular biology of viral transformation, including techniques for cell culturing and viral vector construction.

transformation and signalling

from an adenovirus infection is the common cold. But adenoviruses do have the molecular machinery to transform a mammalian cell into one with several properties of a cancer cell. And because of their relatively small genomes, adenoviruses offered the opportunity to study the development of cancer cells long before the tools of modern molecular biology became available. 'These days, we have a tremendous number of tools for investigating the different molecules playing a role in cell growth. Yet I believe we can learn more from our model of adenovirus transformation. It can help solve puzzles that could not be easily solved otherwise. For instance, the adenoviral antigen E1A binds to a large number of proteins, one of which is the RB protein, the retinoblastoma protein, which is one of the most important tumour suppressor proteins, involved in many types of cancer. Research on the interactions between RB and E1A has led to a better understanding of RB function.'

'These days, we have a tremendous number of tools for investigating the different molecules playing a role in cell growth. Yet I believe we can learn more from our model of adenovirus transformation.'

The MDMX gene

The p53 gene, arguably the most well-known tumour suppressor gene, also came to light through research on viral oncogenes. Van der Eb explains: 'To effect malignant transformation in cells, viruses like adenovirus and SV40 have to somehow bypass p53. If you trigger major changes in the biological behaviour of a cell, p53 will become activated and either arrest the cell cycle or induce apoptosis. We found out that the adenoviral E1B protein

LEX VAN DER EB, LEIDEN UNIVERSITY MEDICAL CENTER

10 key publications

1 Kast, W. M., R. Offringa et al (1989). Eradication of adenovirus E1-induced tumors by E1A-specific cytotoxic T lymphocytes. Cell 59:603-614.

2 Offringa, R., S. Gebel et al (1990). A novel function of the transforming domain of E1a: repression of AP-1 activity. Cell 62:527-538.

3 Van Dam, H., R. Offringa et al (1990). Differential effects of the adenovirus E1A oncogene on members of the AP-1 transcription factor family. Mol. Cell Biol. 10:5857-5864.

4 Van den Heuvel, S. J., T. van Laar et al (1990). Association between the cellular p53 and the adenovirus 5 E1B-55kd proteins reduces the oncogenicity of Ad-transformed cells. EMBO J. 9:2621-2629.

5 Weeda, G., R. C. van Ham et al (1990). A presumed DNA helicase encoded by ERCC-3 is involved in the human repair disorders xeroderma pigmentosum and Cockayne's syndrome. Cell 62:777-791.

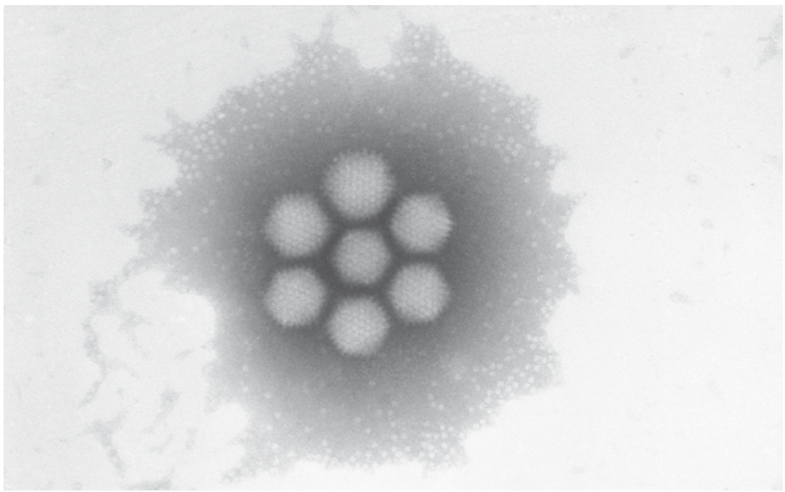
6 Van Dam, H., M. Duyndam et al (1993). Heterodimer formation of cJun and ATF-2 is responsible for induction of c-jun by the 243 amino acid adenovirus E1A protein. EMBO J. 12:479-487.

7 Hoeben, R. C., F. J. Fallaux et al (1995). Expression of the blood-clotting factor-VIII cDNA is repressed by a transcriptional silencer located in its coding region. Blood 85:2447-2454.

8 Schouten, G. J., A. J. van der Eb, and A. Zantema (1995). Downregulation of MHC class I expression due to interference with p105-NF kappa B1 processing by Ad12E1A. EMBO J. 14:1498-1507.

9 Shvarts, A., W. T. Steegenga et al (1996). MDMX: a novel p53-binding protein with some functional properties of MDM2. EMBO J. 15:5349-5357.

10 Danen-van Oorschot, A. A., D. F. Fischer et al (1997). Apoptin induces apoptosis in human transformed and malignant cells but not in normal cells. Proc. Natl. Acad. Sci. U. S. A 94:5843-5847.



Electron micrograph of Adenovirus type 5 particles. The photo was made in the Laboratory of Electron Microscopy (Leiden) in 1967 for Van der Eb's thesis. This virus played a central role for more than 40 years in Van der Eb's research on cell transformation and gene therapy.

forms a stable complex with p53, which can be demonstrated within the transformed cell. That's why p53 became an important focus of our research. One of the regulators of p53 is MDM2. It is involved in a feedback loop with p53. Working as a transcription factor, p53 increases the production of the MDM2 protein, which in turn causes degradation of p53 by ubiquitinating it so it is then targeted for degradation by the proteasome. Jochemsen in our group searched for genes that looked like MDM2. He found a gene he called MDMX, which also is a regulator of p53, although it is not involved in the feedback loop described above. It has some of the same functions as MDM2 as well as some opposing functions. The balance between MDM2 and MDMX seems to be important for keeping p53 in the cell at an optimal level. As we know, too much p53 interferes with the normal cell cycle and can even induce apoptosis, but if there is no p53 there is a danger of malignant growth.'

Gene therapy

Another line of enquiry that seemed promising when the CBG was founded was the investigation of a viral protein called Apoptin, discovered by Matthieu Noteborn of Van der Eb's group. It is produced in cells infected by a DNA virus, the chicken anaemia virus, and causes apoptosis in fast-growing cells – including cancer cells. The Apoptin gene seemed to be a promising candi-

Van der Eb is quite optimistic about the future of gene therapy: 'I believe we will see progress in the coming years, although much more slowly than we hoped for in the early 1990s.'

date for cancer gene therapy. Van der Eb recalls: 'When it became clear that this compound could be used in cancer therapy we immediately applied for a patent. Unfortunately the start-up company that was developed as a result never became fully fledged, but I still believe the concept was scientifically very interesting.'

Rollercoaster ride for viral gene therapy

Since then the use of viral vectors for gene therapy has been a bit of a rollercoaster ride as far as public appraisal goes. After the initial high hopes for the therapy, there was a severe backlash when a patient treated with an adenovirus vector died of multiple organ failure. The alternative form of therapy – using retroviruses instead of adenoviruses – also became problematic when two patients out of ten in a study treated for severe combined immunodeficiency (SCID) developed a leukaemia-like condition. It was found that the retrovirus interfered with the function of the LMO2 gene, inducing malignant transformation. However, new protocols and ways of vector construction can circumvent such problems. Van der Eb is quite optimistic about the future of gene therapy: 'I believe we will see progress in the coming years, although much more slowly than we hoped for in the early 1990s. The failures and setbacks it has experienced show that we need to know a lot more than we did. Now the concepts can be developed again, interacting closely with basic research laboratories. Basically I see two fields of application. First of all, there are genetic disorders, although there is a problem inducing lasting gene expression in target cells (what we often see is a gradual reduction in expression). Then there is the treatment of cancer, in which even short-term expression of a gene can be beneficial. This means that modified adenoviruses can be used as vectors of a therapeutic gene, that will not become integrated in the genome of the cell. Many cancer cells do not have a receptor or sufficient receptors for adenoviruses, therefore the viral capsid would have to be modified so that it can attach to receptors present on the surface of the cancer cell. I find it interesting that the adenoviruses I started studying half a century ago in order to understand more about cancer might now be useful for curing it.'

HANS GEUZE, UNIVERSITY MEDICAL CENTER UTRECHT

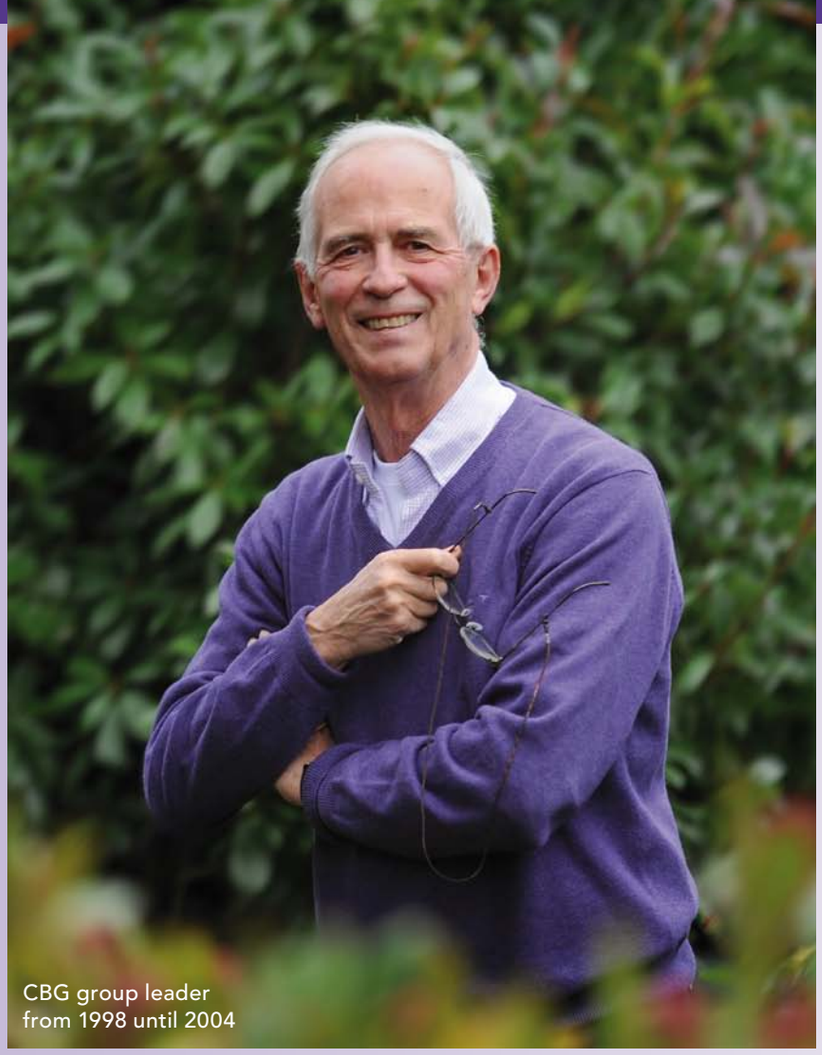
CV

Hans Geuze studied biology at the Free University of Amsterdam where he also wrote a thesis on the morphology and physiology of the equilibrium organs in snails. In 1967 he became Head of the Medical Center of Electron Microscopy in Utrecht and from 1977 until his retirement in 2001 he was research professor at the Department of Cell Biology of the Medical Faculty and later the University Medical Center in Utrecht. Over the years he (co-) chaired and organised many conferences such as the ESF conference on Endocytosis (1993, Como) and the Gordon Conference on Lysosomes (1996, 1998 New Hampshire, USA). He was editor of the J. Cell Biol., Eur. J. Cell Biol. and Biol. Cell. In 1999, he was elected member of the Royal Netherlands Academy of Arts and Sciences (KNAW) and served the KNAW in several committees including that for Medicine. He was board member of International Federation of Societies for Electron Microscopy, co-founder of the Institute of Biomembranes, Utrecht University. In 2001 he was honoured as Knight in the Order of the Dutch Lion.

Where do things happen in the cell?

To understand how a cell works it takes more than knowing exactly *what* happens in the cell. It is also necessary to know *where* it happens in the cell. By developing new techniques like immunolabeling electron microscopy (EM) combined with EM tomography, Hans Geuze – member of the CBG until his retirement in 2003 – was able to discover how and *where* exogenous antigens are processed in antigen-presenting cells before being presented to helper T cells, and that peroxisomes are formed at the endoplasmic reticulum. As he stated, 'Only by integrating morphological, biochemical and molecular data will we eventually be able to develop a coherent picture of the living cell.'

When the CBG began, Geuze felt a bit like an outsider. 'Most of the other members were geneticists, biochemists or molecular biologists. Their primary field of interest was to elucidate the genes and proteins involved in particular cellular functions or in particular diseases. I was the only cell biologist among them who studied the functions of gene products at a higher organisational level – that of cellular organelles. Since organelles contain and are surrounded by membranes, my studies involved protein localisation and membrane structure. My main interest is what happens where in the cell. Therefore you have to focus on the details in the morphology of the cell. To function properly proteins do not only have to be formed correctly, which means that the genes coding for the proteins must be intact and must be transcribed and translated correctly, but the proteins also have to be transported to the correct place in the cell, at the correct time and, in the meantime, be post-translationally modified. Of



CBG group leader
from 1998 until 2004

Goal

To use cryo-immunogold- and electron tomographical techniques to study the processing and loading of antigenic peptides onto MHC molecules and on the biogenesis of peroxisomes.

Facilities and expertise

Facilities of the Centre for Electron Microscopy (CEM): cryo-ultramicrotomes, light-, fluorescent- and electronmicroscopes, a live-cell imaging station, as well as a high-pressure freezer, a critical point dryer and a freeze substitution apparatus.
Expertise: high resolution multiple immunogold labeling of ultrathin cryosections of cells and tissues. This technique allowed for quantitation of proteins at the subcellular level. Recently, electron tomography was implemented in the CEM.

course, the proteins then have to be removed from that place and subsequently carefully dismantled at the proper time as well. This requires a constant and carefully orchestrated transport of proteins through the cell. If even a minor detail in this cascade of events fails (for example, a receptor protein may not appear on the outer membrane of the cell) then the cell could go 'off the rails' and cause disease. My motivation for cooperation within the CBG was to integrate the genetic, biochemical and molecular data about cell functioning obtained by other members with our data about the transport of proteins through the cell, to develop a coherent picture of the living cell.'

'Our finding that the endosome contains small vesicles with MHC class II molecules has led to a patent to use those vesicles for immunotherapy in cancer.'

How and where are antigens processed?

Transport of proteins through the cell is mainly performed by means of intracellular vesicles. These transport vesicles selectively incorporate subsets of proteins (and lipids) from a donor organelle of the cell and deliver their cargo to an acceptor organelle. Geuze says: 'Studies of this process require coordinated morphological, biochemical, and molecular approaches. For this purpose our laboratory has developed immuno-electron microscopical methods that allow us to locate cargo proteins, labelled by antibodies, at nanometre resolution'. One of the topics Geuze has worked on in recent years that uses this technique is the processing of antigens in antigen-presenting cells. 'The processing of antigens that have been taken up by an antigen-presenting cell like a lymphocyte or dendritic cell takes place in the so-called endocytic pathway of the cell. This system is composed of functionally and physically distinct membrane-bound compartments. Early endosomes are responsible for dissociation and sorting of receptors and ligands in an environment that does not damage the receptors intended for re-utilisation. Late endosomes and lysosomes are responsible for accumulation and digestion of exogenous and endogenous macromolecules such

HANS GEUZE, UNIVERSITY MEDICAL CENTER UTRECHT

Key publications

1 Martinez-Menarguez, J., H.J. Geuze et al (1999). Vesicular tubular clusters between ER and Golgi mediate concentration of soluble secretory proteins by exclusion from COPI-coated vesicles. Cell, 98, 81-90.

2 Denzer, K, M.J.Kleijmeer et al (2000). Exosome: from internal vesicle of the multivesicular body to intercellular signaling device. J. Cell Sci. 113, 3365-3374.

3 Oprins, A., C. Rabouille et al (2001). The ER to Golgi interface is the major concentration site of secretory proteins in the exocrine pancreatic cell. Traffic 2, 831-838.

4 Kleijmeer, M., G. Ramm et al (2001). Reorganization of multivesicular bodies regulates MHC class II antigen presentation by dendritic cells. J. Cell Biol. 155, 53-63.

5 Stoorvogel, W., Melief, C.J., Rabouille, C., Geuze, H.J. (2001). Antigen loading of MHC class I molecules in the endocytic pathway. Traffic 2, 124-137.

6 Martinez-Menarguez, J. A., R. Prekeris et al (2001). PeriGolgi vesicles contain retrograde but not anterograde proteins consistent with the cisternal progression model of intra-Golgi transport. J Cell Biol. 155: 1213-1224.

7 Doray, B, Ghosh, P et al (2002). Cooperation of GGAs and AP-1 in Packaging MPRs at the Trans-Golgi Network Science Sep 6 2002: 1700-1703.

8 Gosh, P., Griffith, J. et al (2003). Mammalian GGAs act together to sort mannose 6-phophate receptors. J. Cell Biol. 163, 755-766.

9 Geuze, H.J., Murk, J.L. et al (2003). Involvement of the endoplasmic reticulum in peroxisome formation. Mol. Biol. Cell 14, 2900-2907.

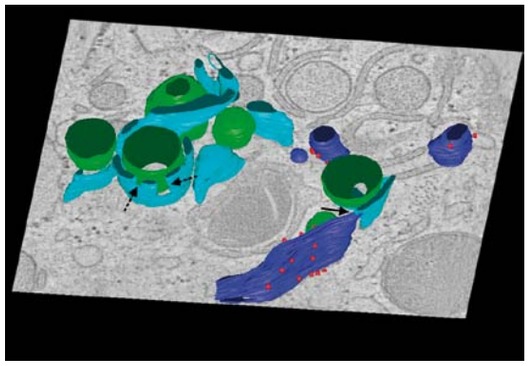
10 Slot, J.W., Geuze, H.J. (2007). Cryosectioning and Immunolabeling. Nature Protocols 2: 2480-249.

as antigens. Using morphological and biochemical methods, along with immunoassays, on a variety of antigen-presenting cells, we have found that late endosomes and lysosomes – collectively called MHC (major histocompatability complex) class II compartments, or MIICs – are the organelles within which endocytosed antigens are processed into peptides that are bound to newly synthesised MHC class II molecules. The fact that lysosomes are involved in the processing of antigens came as a complete surprise for us. It was always believed that the lysosomes' only function was degradation of 'waste'. Now we saw that the lysosome also contains MHC class II molecules and is able to construct a new, functional protein complex – an MHC class II molecule loaded with a peptide of an antigen. Once loaded with peptide, the MHC class II molecules are transported to the surface of the antigen-presenting cell by reorganisation of the MIIC. In addition, we very recently elucidated (in collaboration with our colleges in Leiden) the pathway in dendritic cells that leads to MHC class I-assisted antigen presentation. We showed that dendritic cells contain special lysosome-like organelles, distinct from MIICs, in which antigens can be stored for many days. These storage compartments serve as an antigen source for a continuous supply of MHC class I ligands. This mechanism ensures sustained cross-presentation of antigen by the dendritic cells and priming naive CD8+ T lymphocytes, despite the short-lived expression of MHC class I–peptide complexes at the cell surface. This new knowledge about how and where antigens are processed in antigen-presenting cells could eventually lead to improvements in vaccination strategies. In fact our finding that the endosome contains small vesicles with MHC class II molecules has led to a patent to use those vesicles for immunotherapy in cancer.'

Organelles in three dimensions

Another milestone reached in recent years by Geuze's group is the reconstruction of organelle structure in three dimensions by EM tomography. Geuze: 'This method is conceptually related to other tomographic techniques such as computer tomography as used in a clinical context. We for the first time combined EM tomography with immune-EM, which enabled us to construct a three-dimensional image of certain organelles in

‘My motivation for cooperation within the CBG was to integrate the genetic, biochemical and molecular data about cell functioning obtained by other members with our data about the transport of proteins through the cell, to develop a coherent picture of the living cell.’



Visualization of peroxisome formation. From numerous 4-nm thin EM tomographic slices like the one shown in grey, a 3-D reconstruction is made of the membrane compartments present in a certain cell volume including peroxisomes (green), endoplasmic reticulum (ER, blue) with attached ribosomes (red) and lamellae interconnecting the ER and peroxisomes. Places are indicated where the membranes between ER and lamellae (solid arrow) and between lamellae and peroxisomes (dotted arrows) are continuous.

combination with the localisation of proteins. By doing so, we were able to answer the longstanding question of how and where peroxisomes are newly formed in the cell. Using EM tomography we showed that peroxisomes are formed at the endoplasmic reticulum. Furthermore, this technique enabled us to get a three-dimensional image of the Golgi complex. The ‘circles’ that are observed in traditional two-dimensional EM appeared in fact to be aspects of twisting tubules when looked upon in three dimensions. Although this knowledge is merely ‘textbook knowledge’ a better understanding of how cell organelles (in this case the Golgi complex) are shaped and organised in the cell is very important for understanding their functioning. Cellular activities such as protein sorting, membrane trafficking and signal transduction pathways are usually described in two dimensions, in flat flowcharts. However, depicting these activities in three dimensions could lead to more realistic and more comprehensive views of these cellular activities.’

As he stated previously, Geuze’s motivation to cooperate within the CBG was to integrate the genetic, biochemical and molecular data about cell functioning with morphological data: ‘I have to admit that this was until recently not so successful. This was in part due to technical difficulties which made it impossible to localise the molecules that other members of the CBG were interested in. However, the group that I led until a few years ago is now studying signal transduction systems like Rap and Wnt at the level of the organelle. I expect that in the near future the integration I hoped for at the start of the CBG will be realised.’

FRANK GROSVELD, ERASMUS MEDICAL CENTER ROTTERDAM

CBG group leader
since 1998

Driven by curiosity – and the urge to

'We want to know how things work', says Frank Grosveld. But in the course of the conversation, another of his drives becomes clear: to find ways to cure or prevent hereditary haemoglobin disorders. No one can accuse Grosveld of picking an easy target here; the regulation of haemoglobin synthesis is notoriously difficult. It has forced the Rotterdam team to come up with many creative solutions to nature's challenges – from an innovative way for studying spatial relationships in the nucleus of cells to the production of llama-like antibodies in mice.

'These are very interesting times for anyone working in the life sciences', says Grosveld. 'When I started as a PhD student, you could know almost everyone in the field. These days, it is hard to keep up with developments within your own niche. We experienced the same expansion and the same revolutionary spirit as the sciences of physics and chemistry about a century ago. If you look around, you can see how those scientific breakthroughs of the past have shaped our society. Nobody, especially not the politicians funding research, could have foreseen the enormous impact of physics on our daily lives. The story goes that Faraday, the pioneer of electricity, was asked by the famous politician William Gladstone, the then minister of Finance, the following question: 'All of this is very well, this electricity, but what's the use of it?'. Faraday, who was better at public relations than most current-day scientists, famously replied: 'One day, sir, you may tax it'. And although no one can predict the

CV

Frank Grosveld obtained a PhD at the University of Amsterdam and a PhD at McGill U (1976) followed by two postdocs before becoming Head of Division (1981) at the National Institute for Medical Research, UK. In 1993 he accepted the Chair of Cell Biology at the Erasmus University Rotterdam. He is a Royal Netherlands Academy of Sciences (KNAW) Professor. Mechanisms of gene regulation and epigenetic phenomena are the primary focus of his research. His group has carried out pioneering work in many aspects of gene regulation, discovering; that DNA methylation inhibits gene expression via an indirect mechanism; the first LCR; the first visualization of the primary transcription process in the nucleus; looping between regulatory sequences *in vivo*; the first isolation of a suppressed *in vivo* promoter from the nucleus. He has filed many patents and has closely collaborated with industrial partners. He is a member of the Royal Netherlands Academy of Sciences and a Fellow of the Royal Society (UK). He has been an advisor to a number of companies and is co-founder of five start-ups.

Goals

Unravelling the network of transcription factors that regulate the hematopoietic pathway from the early stem cell to fully differentiated erythroid cells. Understanding the mechanism of long range transcriptional regulation. Unravelling the transcriptional regulation of the ß globin loci with the goal of developing novel therapeutic approaches for the treatment of sickle cell anaemia and thalassemia. Apply/commercialize new discoveries.

Facilities and expertise

Proteomics: equipped with several mass spectrometers and an expert group running the facility. Genomics: equipped with a diverse set of machinery of which the array and massive parallel sequencing technologies are the most important. The output has been coupled to an efficient bio-informatics platform. Imaging: a number of microscopes are operational within this facility, ranging from confocal microscopes to TIRF and 4π microscopy.

cure haemoglobinopathy

outcome of a single experiment, I think it is safe to predict that the current revolution in the life sciences will have a great impact on society in the next decade. In other words, it is more than worth the taxpayers' money, as long as this money is invested in high-quality science. That's what I like about the CBG: it allows scientists the freedom to develop long-term projects that are almost impossible to finance otherwise. We have been doing very successful research projects with CBG money that I would not even dare to propose to other funds for fear of being called fanciful, or worse.'

'When I seriously embarked on this, with a totally novel approach, nobody wanted to finance such a high-risk programme. That is why I'm very grateful to the CBG.'

Switch back to fetal haemoglobin?

The main subject of Grosveld's research is gene regulation, particularly the regulation of globin genes, which are responsible for the protein part of the oxygen transporting haemoglobin. By changing the regulation of the different globin genes, Grosveld and his co-workers hope to establish a cure for the most common hereditary diseases – so-called haemoglobinopathies like sickle cell anaemia and thalassaemia. About 6 percent of the world's population are (hetero-zygous) carriers of one of the haemoglobinopathy genes. Every year 300,000 babies are born with a homozygous haemoglobinopathy. About half of them die within the first few months of their life.

Haemoglobinopathies are prevalent in countries with a high incidence of malaria. The heterozygous carriers of an aberrant globin gene have a higher

FRANK GROSVELD, ERASMUS MEDICAL CENTER ROTTERDAM

Key publications

1 De Krom, Mariken., van de Corput, Mariette., et al (2002). Stochastic patterns in globin gene expression are established prior to transcriptional activation and are clonally inherited. Molecular Cell, 9(6):1319-26.

2 Tolhuis, Bas., Palstra, RobertJan., et al (2002). Looping and interaction between hypersensitive sites in the active ß-globin locus. Molecular Cell, 10:1-20.

3 Palstra, RobertJan., Tolhuis, Bas., et al (2003). The beta-globin nuclear compartment in development and erythroid differentiation. Nat Genet. 2003, 35(2):190-4.

4 Patrinos, George., de Krom, Mariken., et al (2004). Multiple interactions between regulatory regions are required to stabilise an Active Chromatin Hub. Genes & Dev. 18(12):1495-509.

5 Rodriguez Patrick, Bonte Edgar, et al (2005). GATA-1 forms distinct activating and repressive complexes in erythroid cells. EMBO J. ;24(13):2354-66.

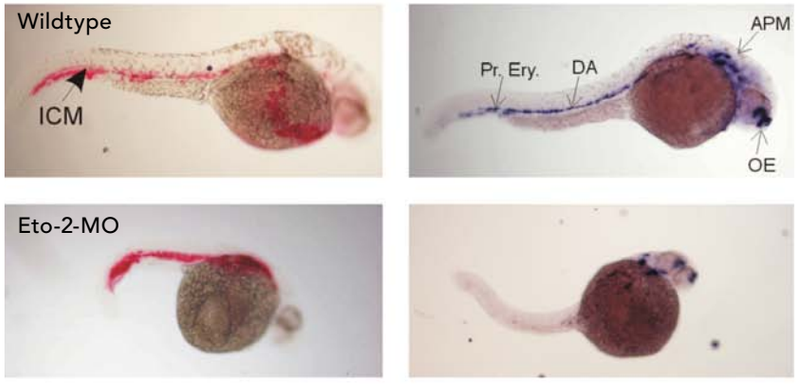
6 Janssens Rick, Dekker Sylvia, et al (2006) Generation of heavy-chain-only antibodies in mice. Proc Natl Acad Sci USA. 2006; 103(41):15130-5.

7 Splinter Erik, Heath Helen, et al (2006). CTCF mediates long-range chromatin looping and local histone modification in the beta-globin locus. Genes Dev. ;20(17):2349-54.

8 Monkhorst Kim, Jonkers Iris, et al (2008). X inactivation counting and choice is a stochastic process: evidence for involvement of an X-linked activator. Cell ;132:410-21.

9 Jonkers I., Tahsin Stefan Barakat, et al (2009). Nrf12 is a dose dependent X-encoded activator of X chromosome inactivation. Cell.;139:999-1011.

10 Soler E., Charlotte Andrieu-Soler, et al. The Genome Wide Dynamics of the Binding of the Ldb1 Complex during Erythroid Differentiation. Genes and Dev. In press.



Analysis of zebrafish embryos after morpholino injections. Top two panels show non injected WT control embryos (30 hours post fertilisation) stained for embryonic ß-globin (left column) and runx1 (right column). The intermediate cell mass (ICM), dorsal aorta (DA), primitive erythrocytes (Pr.Ery.), anterior paraxial mesoderm (APM.) and olfactory epithelium (OE.) are indicated. The globin signal is red, the runx1 signal is blue. The second row of panels are representative embryos injected with a morpholino against the transcription factor Eto2 which was discovered to be part of a large transcription factor complex in hematopoietic cells (Meier et al. (2006) Devel. 133:4913-23). The result shows that the Eto2 is essential for the formation of the definitive (absence of blue staining), but not the primitive hematopoietic system (red staining).

chance of surviving a malaria infection, thus keeping the gene in the gene pool. Most malaria-affected countries are in the developing world, so the medical possibilities are limited. Bone marrow transplants, for instance, may cure a patient, but are far too complex and expensive to use routinely.

Grosveld explains his aims: 'What we hope to achieve, is to selectively switch on the gamma-globin genes in patients. They enable the fetus to make its own haemoglobin with an even higher affinity for oxygen. At birth, the precursors of the red blood cells switch to beta-globin. The gamma-globin genes are permanently blocked. If only we could reactivate gamma-globin expression in adults – we'd have a very elegant and attractive treatment. But when I seriously embarked on this , some eight years ago, with a totally novel approach, nobody wanted to finance such a high-risk programme. That is why I'm very grateful to the CBG. Now, we are preparing a publication describing the protein complex that blocks the transcription of gamma-globin genes in adult bone marrow cells – for us, and hopefully for the patients, an important milestone'.

Spatial relationships

The search for the Holy Grail of gamma-globin re-activation continues, and it has been a strong

Current group members

Senior staff
Elaine Dzierzak
Niels Galjart
Gert Jansen
Sjaak Philipsen
Bob Scholte
Anna Akhmanova

Junior staff
Dubravka Drabek
Tobias Knoch
RobertJan Palstra
Eric Soler

Postdocs
Mariette van de Corput
Ali Imam
Alex Maas
Athna Mylona
Rik Rademaker
Erikjan Rijkers
Charlotte Soler-Andrieu

PhD students
Ali Aghajanirefah
Tiago Brazao
Alireza Ghamari
Ruud Jorna
Petros Papadopoulos
Farzin Pourfarzad
Boet van Riel

Technicians
Ernie de Boer
Rien van Haperen
Rick Janssens
Mary Stevens

drive to remain on the cutting edge of science, to use all the tools of proteomics and bioinformatics and even to develop new techniques in molecular biology. 'Technology is very important to us', says Grosveld. An example is the chromatin conformation capture (3C) technique, originally developed in Boston to study yeast DNA and adapted by the Rotterdam team to understand how the beta-globin gene is regulated by its locus control region (LCR), which is located up to 40–60 kilobases away from the gene itself. They demonstrated that in the nucleus of red blood cell precursors, the LCR is in proximity to the ß-globin genes. Grosveld continues: 'Now this technique is used worldwide to establish spatial relationships between different parts of the genome that may be far apart if you think of the DNA in linear terms. With 3C, and its successor 3C-on-chip or 4C, we 'freeze' the interactions between different parts of the DNA and show how they stick together in reality.'

Of mice and men – and llamas

Scientific imagination and Grosveld's practical mind are also at the basis of a new spin-off company with a high potential for success. Harbour Antibodies produces so-called heavy-chain antibodies. Grosveld describes the rationale behind this: 'We needed these antibodies for our research. By coincidence, I heard of the antibodies made by llamas and other members of the camel family, that consist only of heavy chains. For a time we even kept llamas for their antibodies. But then it occurred to me that it would be easier to have mice that produce these antibodies. Furthermore, we wanted human heavy chains – not llama ones. So we set out to do that, and we did it and we patented all the techniques.' Interestingly, this whole endeavour may also benefit potential carriers of haemoglobinopathy genes. Heavy-chain antibodies can be used in screening kits for the detection of carriers. If both parents know they are carriers, they can seek help at a prenatal diagnostics centre. Grosveld summarises: 'Haemoglobinopathy will always be in the back of my mind, even if we pursue many other paths in our scientific curiosity'.

ALBERT HECK, UTRECHT UNIVERSITY

CBG group leader since 2009

Global view of the 'cellular society'

Mass spectrometry-based, large-scale analysis and characterisation of proteins has become an elementary technique in the life sciences during the last decade. Albert Heck in Utrecht is one of the leading scientists in the world within this field. Driven by his fascination for the complexity of the living cell, he focuses on the development of new techniques to analyse and characterise proteins in all their facets, so that 'eventually we will be able to understand how a living cell really works at the molecular level and what goes wrong in disease'.

Although among the 'youngsters' of the CBG (his group only joined the CBG officially in 2009) Albert Heck has been linked with the CBG since he came to Utrecht in 1998 as a professor of biomolecular mass spectrometry. 'Right from the start, CBG significantly invested in the introduction and further development of mass spectrometry-based proteomics. Mass spectrometry is an analytical technique for analysing and characterising very quickly minute amount of proteins, and it has evolved enormously during the last decade. Now it is considered as an essential proteomics technique that will help us build a more detailed understanding of how a cell works.'
It is precisely that search – for a more detailed understanding of how cells work – that motivates Heck. 'I am fascinated by the complexity and beauty of the living cell at the molecular level. In particular by how those tens of thousands of different proteins that are present in every cell work so harmoniously together that a cell is able to

CV

Albert Heck is professor at the Science Faculty of Utrecht University. The general theme of the research in his group is to develop and implement innovative mass spectrometric methods for the more efficient and detailed characterization of proteins in relation to their biological function. The Heck group is strong in the development of proteomics enabling technologies (e.g. protein expression analysis, post-translational modifications and protein-interaction networks) and in macromolecular native mass spectrometry, which focuses on the analysis of intact protein machineries. Heck is scientific director of the Netherlands Proteomics Centre (since 2003) and the Bijvoet Centre for Biomolecular Research (since 2006). He is member of the council of the Human Proteome Organization (HUPO). In 2001 he was recipient of the Golden Medal of the Dutch Royal Chemical Society. In 2004 he received an honoree Utrecht University "ABC"-professorship and in 2006 the Descartes-Huygens award from the "Académie des Sciences" of France. He has currently (co-)authored more than 250 papers in internationally reviewed journals.

Goals

The development of proteomics enabling technologies. Through interactions with other CBG groups translating these advanced enabling tools efficiently into investigations into stem cell biology, aging and cancer. To identify key markers for biological (mal)functioning.

Facilities and expertise

Through Heck's participation in the CBG, members have access to one of the most advanced proteomics infrastructures and concomitant analytical, bioinformatics and biochemistry expertise in Europe. The infrastructure includes over fifteen mass spectrometers and an expert group of about forty people.

respond, for example, to the entrance of a virus, and can also duplicate itself, or migrate throughout the body. The 'cellular society' is like human society; it only works properly if the right people are at the right place at the right time and they work together smoothly. It is often also a game of give and take. If you want to understand how a particular process in the living cell is performed properly, you first have to identify all the members of the cellular 'society' involved in the process and how they are interconnected. Conversely, if you want to understand why a particular process in a cell goes wrong, you have to identify which members are *not* at the right place at the right time, or don't work together properly with the other members.'

'That is obviously one of the strengths of the CBG – all its members share their expertise and they support and inspire each other in finding new ways to answer fundamental biological and clinical questions.'

First glimpse of massive protein networks

Heck states: 'Currently mass spectrometry enables us to study about a thousand proteins in a cell simultaneously. This gives us the opportunity to study proteins in their cellular context and to monitor a network of interconnected proteins in action. Evidently, a thousand proteins is still only the tip of the iceberg. But it enables us to catch a *first glimpse* of what really happens in the cell. The main goal of our group is to further develop proteomics technologies, to refine our techniques, to make them even more sensitive, faster and more comprehensive, not only to analyse more proteins, but also to look more precisely at what these proteins are doing in the cell and how they do what they do. With these refined techniques we often collaborate with other groups to determine what sorts of proteins are likely to be involved in the process they are studying. That is why close collaboration with the other groups in the CBG is, for us, so important. Our proteomics technologies provide a core methodology that can be used by other

ALBERT HECK, UTRECHT UNIVERSITY

Key publications

1 E. van Anken, E. P. Romijn et al (2003). Sequential waves of functionally related proteins are expressed when B cells prepare for antibody secretion. Immunity 18; 243-253.

2 J. Krijgsveld, R. F. Ketting et al (2003). Metabolic labeling of C. elegans and Drosophila for quantitative proteomics. Nature Biotech. 21; 927-931.

3 M. W. H. Pinkse, P. M. Uitto et al (2004). Selective isolation at the femtomole level of phosphopeptides from proteolytic digests using 2D-nanoLC-ESI-MS/MS and titanium oxide pre-columns. Anal. Chem 76; 3935-3943.

4 A. Kolkman, E. H.C. Dirksen et al (2005). Double Standards in Quantitative Proteomics: Direct comparative assessment of Difference in Gel Electrophoresis (DiGE) and metabolic stable isotope labeling. Mol. Cell. Proteomics 4; 255-266.

5 R. H. H. van den Heuvel, E. van Duijn et al (2006). Improving the Performance of a Quadrupole Time-of-Flight Instrument for Macromolecular Mass Spectrometry, Anal. Chem. 78; 7473-7483.

6 M. W. H. Pinkse, S. Mohammed et al (2008). Highly robust, automated on-line TiO2 based phosphoproteomics at attomole sensitivity applied to endogenous phosphorylation. J Proteome Res 7; 687-697.

7 C. Uetrecht, C. Versluis et al (2008). High Resolution Mass Spectrometry of Viral Assemblies: Molecular Compositions and Stability of Dimorphic Hepatitis B Virus Capsids. PNAS USA 105; 9216-9220.

8 N. Taouatas, M. M. Drugan, A. J. R. Heck, S. Mohammed (2008). Straightforward ladder sequencing of peptides using a Lys-N metalloendopeptidase. Nature Methods 5 ; 405-407.

9 A. J. R. Heck (2008). Native mass spectrometry: a bridge between interactomics and structural biology. Nature Methods5; 927-933.

10 D. Van Hoof, J. Muñoz et al (2009). Phosphorylation Dynamics During Early Differentiation of Human Embryonic Stem Cells. Cell Stem Cell 5 ; 214-226.

researchers in addressing a broad array of research questions like: How does a cell move forward in one direction? What makes a stem cell differentiate into a heart muscle cell? Why doesn't a tumour cell stop duplicating? In order to answer all these questions, knowledge is needed about the activity and changes in place and time of the protein networks in the cells. Our group offers state-of-the-art tools and some of the most advanced proteomics infrastructure and expertise in Europe for pinpointing and analysing these networks. For example, working together with Hans Clevers' group we used proteomics techniques to target potentially novel proteins in the Wnt signalling pathway (a pathway involved in the biology of stem cells and self-renewing tissues). Up to that point, classical biochemical research methods had revealed, one-by-one, approximately a dozen proteins involved in this pathway. By using our proteomics techniques we expanded this number rapidly, albeit putatively, to nearly one hundred proteins! Working with experts on Wnt signalling, the members of Hans Clevers' group must decide which of these proteins are likely to be the most important to follow-up. By presenting the refined proteomics techniques developed by our group, we hope to inspire other members of the CBG to use them and employ them efficiently in novel investigations to obtain new answers to specific biological or clinical questions. That is obviously one of the strengths of the CBG – all its members share their expertise and they support and inspire each other in finding new ways to answer fundamental biological and clinical questions. There is a lot of synergy within the CBG.'

Innovative technique for studying protein phosphorylation

Techniques for the global analysis of proteins have become more and more sophisticated in recent years. Heck's group, for example, developed an innovative method for determining whether or not a protein is phosphorylated. As Heck explains: 'Phosphorylation is an essential protein modification to activate a protein, which cannot be easily studied at the genome level. We invested a few years, from 2001 until 2004, in introducing a completely novel method by which phosphorylation of proteins in a cell sample can be measured much more rapidly and sensitively. With this

Current group members

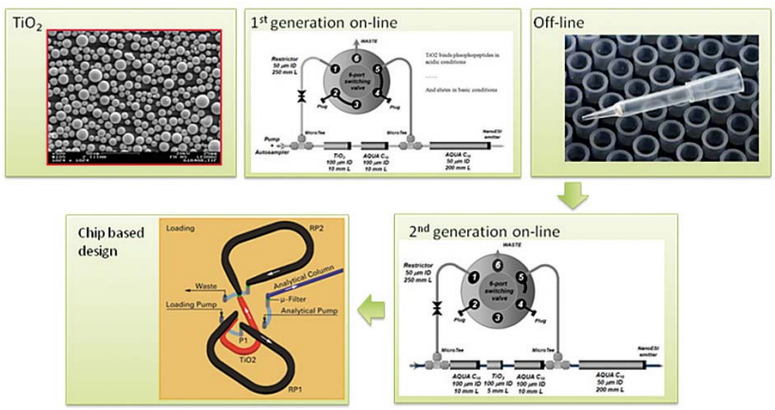
Senior staff
Ron Heeren
Jeroen Krijgsveld (now EMBL)
Monique Slijper

Junior staff
Shabaz Mohammed
Arjen Scholten
Reinout Raijmakers
Maarten Altelaar
Esther van Duijn
Renske van Gestel

Postdocs
Ioana Barbu
Onno Bleijerveld
Salvatore Cappadona
Kristina Lorenzen
Teck Low
Muñoz-Peralta
J.D. Navarro-Herrera
Pieter Neerincx
Sarah Pelletier
Christian Preisinger
Jeffrey Ringrose
Rebecca Rose
Thomas Schwend
Wim Spee
Henk van den Toorn
Houjiang Zhou

PhD students
Thin Thin Aye
Benjamin Barasa
Pepijn Burgers
Serena Di Palma
Christian Frese
Sharon Gauci
Erik de Graaf
Andreas Helbig
Marco Hennrich
Donna Kovanich
Nikolai Mischerikow
Chhiti Pandey
Ayse Polat
Nadia Taouatas
Charlotte Uetrecht
Adja Zoumaro-Djayoon

Technicians
Arjan Barendregt
Mirjam Damen
Soenita Goerdayal
Martina O'Flaherty
Harm Post
Kees Versluis



The Heck laboratory has a track record in proteomics and especially in the analysis of protein post-translational modifications. They introduced TiO2 as enrichment material for the targeted analysis of phosphopeptides, and implemented this technique over the years into a miniaturized on-line automatic system, and on a micro-chip device.

technique, which uses a metal oxide–phosphopeptide affinity enrichment step, it is now possible to identify and monitor in time more than 10,000 different protein phosphorylations in a cell. It enables us not only to determine which proteins are involved in a particular activity of the cell, but also which of these proteins are activated at that particular moment. Together with stem cell researchers we have employed this technique already to explore activation of proteins in the first hour of differentiation in human embryonic stem cells, with the aim of understanding the underlying principles of cellular differentiation. Furthermore, together with a commercial partner we made this novel technique available on a microfluidic chip, so that it can be readily implemented in non-expert laboratories. In a few years the phosphoproteomics technique we developed has become the standard technique in the field for analysing the phosphorylation of proteins.'

The future of proteomics

In the near future Heck hopes to further improve proteomics techniques for analysing and characterising proteins from cell samples. 'A remaining issue in proteomics is that there is still, in many cases, a lot of noise and little signal. Working together with biologists, biochemists, experts in biostatistics, physicists and technicians, we hope to improve the signal and dampen the noise. This requires that experiments are carefully planned within a multidisciplinary context, like that existing within the CBG community. Eventually we will be able to look at the living cell so closely that we will understand how it works at the molecular level – and what goes wrong in disease.'

JAN HOEIJMAKERS, ERASMUS MEDICAL CENTER ROTTERDAM

CBG group leader
since 1998

Understanding ageing from a DNA perspective

When Jan Hoeijmakers and his colleagues published their first papers on ageing, they were highly criticised and even ridiculed. How could a group who specialised in DNA repair and cancer research suddenly claim to be experts on ageing? Now researchers from all over the world want to use their mouse models, based on rare hereditary mutations in the DNA repair machinery. Using advanced biomolecular techniques and a creative approach, they have shown that many aspects of ageing are caused by the accumulation of DNA damage.

'To compete in our field we have to use state-of-the-art technology', says Hoeijmakers. 'Take for instance microarray technology: fifteen years ago, it was cutting edge, but you had to do it all by yourself, tinkering with rare Japanese glass and probes that you had made yourself. Nowadays, you can buy ready-made kits. And now, these are already being replaced by high-throughput sequencing. So we have to invest in this new technology, to be able to keep up with the rest of the world. CBG has helped us to maintain our position in this very competitive field.'

'Participating in CBG is also like having membership of an exclusive club; it helps to get things done, and to attract the right people. And then there is the exchange of people and ideas between our institutions. That is inspiring, both

CV

Jan Hoeijmakers studied biology in Nijmegen. His PhD project (UvA) resolved the molecular basis of antigenic variation by which trypanosomes cause sleeping sickness by switching surface coats. In 1981 he joined the Genetics Dept. (EUR) to work on DNA repair. He cloned many human repair genes allowing elucidation of nucleotide excision repair, resolved the basis of human repair syndromes, identified 'basal transcription disorders', generated many repair mouse mutants and discovered a strong link between DNA damage and aging. Some mutants exhibit dramatically accelerated aging (lifespan ~3 weeks). Conditional mutants allowed targeting accelerated aging to specific organs (e.g. only in brain). Expression profiling revealed an unexpected similarity between short- and long-lived mice: both suppress the somatotrophic axis, as part of a 'survival response' that promotes healthy aging and counteracts cancer by redirecting resources from growth to defences. Additionally, his group explored the dynamic organization of DNA repair in living cells and intact organisms and generated the first mouse mutants without biological clock. His work provided the basis for 'DNage' a company aiming to provide solutions for aging-associated health problems.

Goals

To understand the molecular mechanism of various important DNA damage repair and genome maintenance systems from the single molecule level to the level of living intact organisms including mammals. To elucidate the biological and clinical impact of these systems on cancer, aging and aging-related diseases and inborn disorders. To utilize this knowledge for the prevention of cancer and aging-associated pathology and to promote healthy aging.

Facilities and expertise

Sophisticated single molecule technology (e.g. scanning force microscopy, TIRF), advanced molecular and cell biology (living cell spectroscopy using photo-bleaching technology, e.g. FRAP, FRET, CFS, laser-directed subnuclear damage induction), mouse genetics, high-throughput technology (genomics including massive parallel sequencing, proteomics, metabolomics, bioinformatics) and systems biology.

for senior scientists like myself and for the young PhD students and postdocs who are at the beginning of their careers. You can also make special arrangements, dividing tasks between laboratories, so one group can concentrate on an important part of the problem and the others benefit from their results. Microarray technology is a good example. Our colleagues in Leiden and at the Netherlands Cancer Institute were the pioneers – we could concentrate on other technologies, knowing that they would share their facilities and their practical skills with us.'

Hoeijmakers and his team cloned the first human DNA repair gene. They also discovered the strong evolutionary conservation of DNA repair pathways.

Integrity

'What interests us is one of the most basic problems in biology: how is the integrity of the DNA molecule and the information it contains maintained?' Hoeijmakers continues: 'DNA is at the top of the information hierarchy in the cell. If any other molecule gets damaged, it may have *some* consequences, but after some time this molecule will be replaced by another. Damage to the DNA, however, means loss of information, with long-lasting consequences for the cell and sometimes for the organism as a whole'. Of course DNA is being damaged all the time, due to external influences like ultraviolet light and other sources of ionising radiation, or toxic substances like those in cigarette smoke, as well as internal influences like reactive oxygen species (ROS) and the intrinsic instability of the chemical bonds in the DNA molecule itself. Were it not for the intricate repair mechanisms in every cell, the 'book of life' would soon be unreadable, with dire consequences – the accumulation of mutations leading to cancer and ageing (leading to cell loss and functional decline). Hoeijmakers explains: 'Of course, both cancer and ageing are important topics in modern-day medicine. In an ageing population, more people will get cancer, but also other age-related chronic diseases and conditions of old age such as type 2 diabetes and neurode-

JAN HOEIJMAKERS, ERASMUS MEDICAL CENTER ROTTERDAM

Key publications

1 G.T. van der Horst, M. Muijtjens et al (1999). Mammalian blue-light receptor homologs CRY1 and CRY2 are essential for maintenance of the biological clock. Nature 398, 627-630.

2 A.B. Houtsmuller, S. Rademakers et al (1999). Action of DNA repair endonuclease ERCC1/XPF in living cells. Science 284, 958-961.

3 W. Vermeulen, S. Rademakers et al (2001). A temperature-sensitive disorder in basal transcription and DNA repair in man. Nature Gen. 27, 299-303.

4 Science (research article), 296, 1276-1279. J.H. Hoeijmakers (2001). Genome maintenance mechanisms for preventing cancer. Nature 411, 366-374.

5 J. de Boer, J.O. Andressoo et al (2002). Premature aging in mice deficient in DNA repair and transcription.

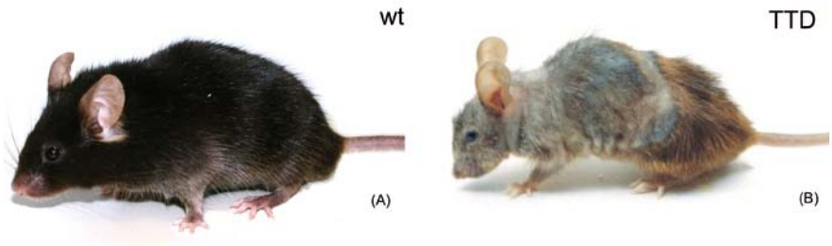
6 G. Giglia-Mari, F. Coin et al (2004). A new, tenth subunit of TFIIH is responsible for the DNA repair syndrome Trichothiodystrophy group A. Nature Genet. 36, 714-719.

7 L.J. Niedernhofer, A.S.Lalai and J.H. Hoeijmakers (2005). Fanconi anemia (cross)linked to DNA repair. Cell 7, 1191-8.

8 L.J. Niedernhofer, G.A. Garinis, et al (2006). A new progeriod syndrome reveals that genotoxic stress suppresses the somatotroph axis. Nature 444, 1038-1043

9 Garinis, G. A., L. M. Uittenboogaard, et al. (2009). "Persistent transcription-blocking DNA lesions trigger somatic growth attenuation associated with longevity." Nat Cell Biol 11(5): 604-15.

10 Hoeijmakers, J. H. (2009). "DNA damage, aging, and cancer." N Engl J Med 361(15): 1475-85.



Transgenic mice engineered to express a mutant DNA helicase show premature aging. The helicase, XPD, is involved in transcription and DNA repair. Compared with a wild-type mouse (A), a transgenic mouse with a defective XPD (B) exhibits many symptoms of premature aging, including osteoporosis, early graying, infertility, and reduced life-span. The mutation in XPD mimics a mutation that in humans causes trichothiodystrophy (TTD), a disorder characterized by brittle hair, skeletal abnormalities, and a very reduced life expectancy. These results indicate that accumulation of DNA damage can contribute to aging in both humans and mice.
(From Molecular Biology of The Cell, Fifth Edition, Alberts et al, Chapter 8, Manipulation Proteins, DNA, and RNA, page 568. Based on a publication by J. de Boer et al., Science 296: 1276-1279, 2002. With permission from AAAS)

> generative disorders like Alzheimer's disease. Our research may not provide all the answers to these problems, but we do hope to gain fundamental insights into the processes of cancer and ageing at the level of cells and macromolecules. This may provide us with better tools to prevent, diagnose and treat the diseases of old age.'

Hereditary diseases

Hoeijmakers and his team of over fifty scientists cloned the first human DNA repair gene. They also discovered the strong evolutionary conservation of DNA repair pathways, unravelled the function of a number of the components, and elucidated the basis of several human DNA repair syndromes. Hereditary syndromes have offered a very fruitful approach to the study of DNA repair, and such 'experiments by Nature' pose a number of questions: What happens if a repair mechanism does not function properly? How do hereditary mutations in DNA repair genes relate to symptoms? At a first look, the relationship between rare genetic defects and clinical symptoms is hard to see. Some of the DNA repair syndromes being studied by Hoeijmakers primarily cause cancer, especially cancer of the skin. Damage caused by ultraviolet light clearly causes many mutations in the DNA, increasing the chance of developing skin cancer to dramatic proportions. But mutations in the very same genes are involved in DNA repair syndromes in which the main symptoms boil down to premature ageing. Zooming in on these genes, it can be shown that mutations in

Current group members

Senior staff
Koos Jaspers, Roland Kanaar, Dies Meijer, Dik van Gent, Bert van der Horst, Wim Vermeulen, Claire Wyman

Junior staff
Ines Chaves - Machado Ferreira, Jeroen Essers, Joyce Lebbink, Martine Jaegle, Hannes Lans, Jurge Marteijn, Filippo Tamanini

Postdocs
Gina Abello, Cintia Bombardieri, Sander Botter, Michele Cristovao, Pauline Ikpa, Takabumi Inagawa, Ed Jacobs, Ricardo Leite, Herve Menoni, Indriati Notorahardjo, Alexandre Ottaviani, Ekim Ozkaynak, Joris Pothof, Dejan Ristic, Shoko Saito, Humberto Sanchez, Maria Tresini, Monique de Waard, Alex Zelensky

PhD students
Eerik Aunin, Ozge Aydin, Kasper Derks, Eugin Destici, Berina Eppink, Klaas Hermans, Thomas Holthausen, Linde Kegel, Eri Kinoshita, Claudia Nicolaije, Romana Nijman, Noorie Noorjahan, Natasja Ramnath, Khek Chian Tham, Ilaria Trezza, Lieneke Uittenboogaard, Iztok Vidic, Daniel Warmerdam, Annelieke de Wit, Maikel Wouters

Technicians
Sander Barnhoorn, Karen Basoalto Ibarra, Cecile Beerens, Renate Brandt, Antonio Carvalho da Silvo, Siska Driegen, Andre Eker, Paula van Heijningen, Roel Janssens, Charlie Laffeber, Hannie Odijk, Anja Raams, Yanto Ridwan, Yvonne. Rijksen, Sari van Rossum-Fikkert, Sigrid Swagemakers, Arjen Theil, Nicole Verkaik, Nils Wijgers, Jan de Wit, Humaira Yousaf

the same domains of the gene may *either* cause cancer or ageing.

Hoeijmakers clarifies: 'These genes contain the code for proteins that form part of a protein complex which is involved in two different kinds of DNA repair. Both of these repair processes involve nucleotide excision, in which a damaged part of a DNA strand is located and cut out. Then it can be synthesised again using the information on the other, non-damaged strand of the double helix. The first process is called "global genome nucleotide excision repair" and with this process the complete genome is constantly monitored and repaired. If this does not function sufficiently, mutations will accumulate and the cell has a high risk of changing into a cancer cell. On the other hand ''transcription-linked nucleotide excision repair" only repairs damage in genes as they are being "read" before being transcribed into messenger RNA. Failure here will cause transcription to stop at the site of a damaged nucleotide. This means that the cell can no longer produce certain proteins and will function worse and worse, or even die. So if a mutation causes damage to the *transcription*-linked form of repair, ageing on both a cell and tissue level ensues. If a mutation primarily affects the *global*-type repair, mutations accumulate so that the cancer risk becomes high.'

Adding life to years

After starting with cancer research, Hoeijmakers and his colleagues have become some of the most prominent researchers on the process of ageing. They have demonstrated important similarities between normal ageing and the accelerated ageing process in transgenic mice carrying the same mutations as patients with DNA repair syndromes. These mouse models, and the expertise of the Rotterdam group, may contribute to the development of new drugs and methods for enhancing the quality of life of older people. To this end, they even founded a company called DNAge, which has been part of Pharming since 2006. Hoeijmakers says: 'As the saying goes, we want to add life to years primarily – not years to lives. But it may well be that the human life span will increase further. It already has changed from around 30 during most of humanity's history to well over 80 years in countries like Japan.'

ROBERT KAPTEIN, UTRECHT UNIVERSITY

CBG group leader
from 1998 until 2008

Adding more cogs to the machine

Information about the three-dimensional structure of bio-macromolecules, like proteins, DNA and RNA, is important for fully comprehending their function in the cell. Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for elucidating this structure. During his career, Rob Kaptein developed and used this technique to determine, among other things, the interaction of components of the nucleotide excision repair system and the way the *lac* repressor binds to DNA. The CBG provided a challenging and stimulating scientific environment for his research.

'To participate in the CBG I have always regarded as an honour', states Rob Kaptein. 'The CBG was, and still is, a research consortium formed by scientists that all have an outstanding track record in the life sciences. To be selected as one of the members and to work together with the other members – until my retirement in 2006 – was indeed an honour, especially for a scientist who is, so to say, working at edge of the main research interests of the CBG.' By this 'edge' Kaptein means structural biology. This area of research aims to elucidate the three-dimensional conformation of biological macromolecules like proteins, DNA and RNA as well as the way these macromolecules interact with each other. The most important tool he uses in this quest is NMR spectroscopy. During his career Kaptein has witnessed the development of this powerful imaging technique, and he played an important part in it. For example, he was the first person to offer a theoretical explanation for CIDNP (chemi-

CV

Robert Kaptein received his PhD in 1971 from Leiden University where he worked on the mechanism of Chemically Induced Dynamic Nuclear Polarization (CIDNP). After a post-doctoral training and a short stay at Shell laboratory in Amsterdam, he went to the University of Groningen as a supervisor of the Dutch national NMR facility. In 1987 he joined Utrecht University as a professor. From 2002 to 2008 he was Secretary General of the Royal Netherlands Academy of Arts and Sciences (KNAW). He is now emeritus professor in Utrecht.

His research deals with NMR spectroscopy applied to problems in structural biology. Early high-lights include the development of the novel Radical Pair theory for CIDNP and the 3D structure of lac repressor headpiece in1985, which was one of the first protein structures determined by NMR. Lac repressor served as a model for in-depth studies of structural and dynamic aspects of protein-DNA recognition and allosteric interactions. Other major projects include the structure of ERCC1/XPF domains (with Jan Hoeijmakers) and the structure and light-induced unfolding of Photo-active Yellow Protein (with Klaas Hellingwerf).

Goal

To understand the mechanisms of gene regulation and DNA repair at a molecular level using the methods of structural biology, in particular NMR spectroscopy.

Facilities and expertise

High-resolution and solid state NMR spectroscopy (spectrometers ranging from 360 to 900 MHz); Coupling lasers with NMR for photobiology and photo-CIDNP research; Protein expression in bacteria (isotope labeling, high-throughput expression using robotics); Computational modeling.

cally induced dynamic nuclear polarisation). Furthermore, it was this technique that brought him in contact with research on biological macromolecules. 'CIDNP is an excellent technique to map the surface of proteins. This provides important information about the mode of interaction between proteins and their ligands.' In time it also became possible to determine the spatial structure of proteins by NMR. Knowing the three-dimensional conformation of these biomacromolecules is necessary to understand how they work and interact *in vivo*, or why a mutation prevents them from functioning properly. By producing 'nice pictures' of biomolecules we enable scientist that are working on DNA repair, for example, to interpret their data in three dimensions. With our research we add more 'cogs' to the machine of biology. Being a member of the CBG ensures that these 'cogs' are applied swiftly and smoothly into the research themes of the other members of the CBG. For example, we worked with Hans Bos's group to improve understanding about the workings of the Rap1-activator Epac; and our research with Peter van der Vliet's group was on the structural characterisation of transcription factors. Another advantage of being a member of the CBG is, of course, the grants, because NMR spectroscopy is very expensive. Fortunately the Netherlands Organisation for Scientific Research (NWO) has always invested generously in this technique in the Netherlands. As a result, the Bijvoet Centre has become an international centre of excellence in structural biology. Additionally, the grants from the CBG have allowed us to keep our equipment up to date and to purchase peripheral equipment.'

'These data complete our view of how transcriptional regulation by means of the *lac* repressor works.'

Specific and non-specific DNA binding

One of the pioneering areas of Kaptein's research was the *lac* repressor system. 'The *lac* repressor system, although part of the prokaryotic cell *Escherichia coli*, has been a central model for understanding transcriptional control since the

ROBERT KAPTEIN, UTRECHT UNIVERSITY

Key publications

1 C.A.E.M. Spronk, G.E. Folkers et al. "Hinge-helix formation and DNA-bending in various lac repressor-operator complexes", EMBO J. 18 (1999) 6472-6480.

2 C.G. Kalodimos, G.E. Folkers et al. Strong DNA binding by covalently-linked dimeric Lac headpiece: Evidence for the crucial role of the hinge helices, Proc.Natl.Acad.Sci. USA 98 (2001), 6039-6044.

3 C.G.Kalodimos, R.Boelens and R.Kaptein. A Residue-Specific View of the Association and Dissociation Pathway in Protein-DNA Recognition. Nature Struct. Biol. 9 (2002),193-197.

4 C.G Kalodimos, A.M.J.J. Bonvin et al. Plasticity in protein-DNA recognition: lac repressor interacts with its natural operator O1 through alternative conformations of its DNA-binding domain. EMBO J. 21 (2002), 2866-2876.

5 C. Dominguez, R. Boelens and A.M.J. J. Bonvin. HADDOCK: A protein-protein docking approach based on biochemical or biophysical information. J.Amer.Chem.Soc. 125 (2003), 1731-1737.

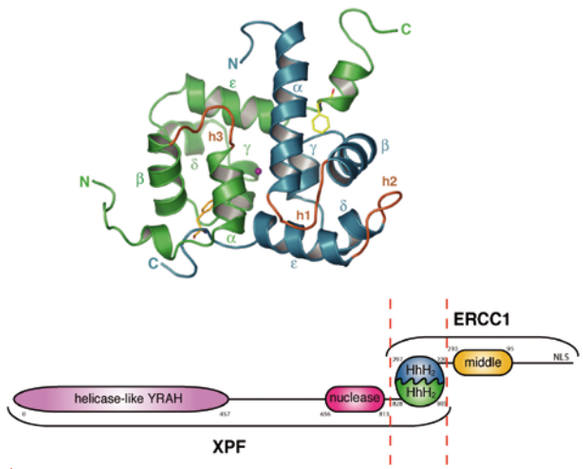
6 S.-T.D. Hsu, E.Breukink et al. The nisin/lipid II complex reveals a pyrophosphate cage that provides a blueprint for novel antibiotics. Nature Struct.Mol.Biol. 11 (2004), 963-967.

7 C.G.Kalodimos, N. Biris et al. Structure and Flexibility Adaptation in Nonspecific and Specific Protein-DNA Complexes. Science 305 (2004), 386-389.

8 C. Bernard, K. Houben et al. The solution structure of a transient photoreceptor intermediate: 25-Photoactive Yellow Protein. Structure 13 (2005), 953-962.

9 K.Tripsianes, G.Folkers et al. The Structure of the Human ERCC1/XPF Interaction Domains Reveals a Complementary Role for the Two Proteins in Nucleotide Excision Repair. Structure 13 (2005), 1849-1858.

10 D. Das, K.Tripsianes et al. The HhH domain of the human DNA repair protein XPF forms stable homodimers. Proteins 70 (2008), 1551–1563.



Structure and domain organisation of ERCC1/XPF

The C-terminal interaction domains of ERCC1 (blue) and XPF (green) contain double helix-hairpin-helix (HhH)2 elements and share a high degree of homology. The complex of these domains (a) shows a quasi 2-fold symmetry axis. The loop h2 of ERCC1 is important for DNA binding at the junction of single and double-stranded DNA. (b) Domain organisation of ERCC1/XPF. In archaebacteria both DNA binding and nucleotide excision are performed by a related XPF homodimer. Ineukaryotes, XPF and ERCC1 evolved from a common XPF ancestor and their domains acquired specialised functions: nuclease activity remained with XPF, while the homologous "middle" domain of ERCC1 lost this activity, yet can bind to XPA which allows ERCC1/XPF to be recruited to the NER complex.

early work done by Jacob and Monod', explains Kaptein. 'This research led to the view of the transcription of genes being under the control of a repressor which switches on gene transcription by binding a specific inducer. To understand this mechanism in detail in the past the three-dimensional structures were elucidated by x-ray studies of the *lac* repressor in its free form, the repressor bound to specific operator DNA target sites and the repressor bound to the inducer. However, the resolution of the X-ray structures was low and our detailed understanding of repressor–DNA binding has come from NMR. Another piece of the puzzle was still missing however – the conformation of the *lac* repressor bound to non-specific sites of the DNA. This non-specific DNA binding of the *lac* repressor is very important for the formation kinetics of the repressor–operator complex. Studies showed that the *lac* repressor is able to find its operator target a hundred to thousand times faster than expected for a diffusion-controlled reaction. This fuelled the idea that non-specific DNA binding of the repressor increases the rate of finding the operator by facilitating transport of the repressor to the operator site. Three mechanisms were proposed to describe this facilitated transport: by sliding along the DNA; by intersegmental transfer; or by hopping along the DNA strand. By elucidating the molecular structure of the repressor bound to non-specific DNA we were able to prove that the sliding model is indeed possible. The repressor is bound to non-specific DNA by means of electrostatic interactions that allow the repressor to move along the strand. When arriving at the operator, conformational changes take place in

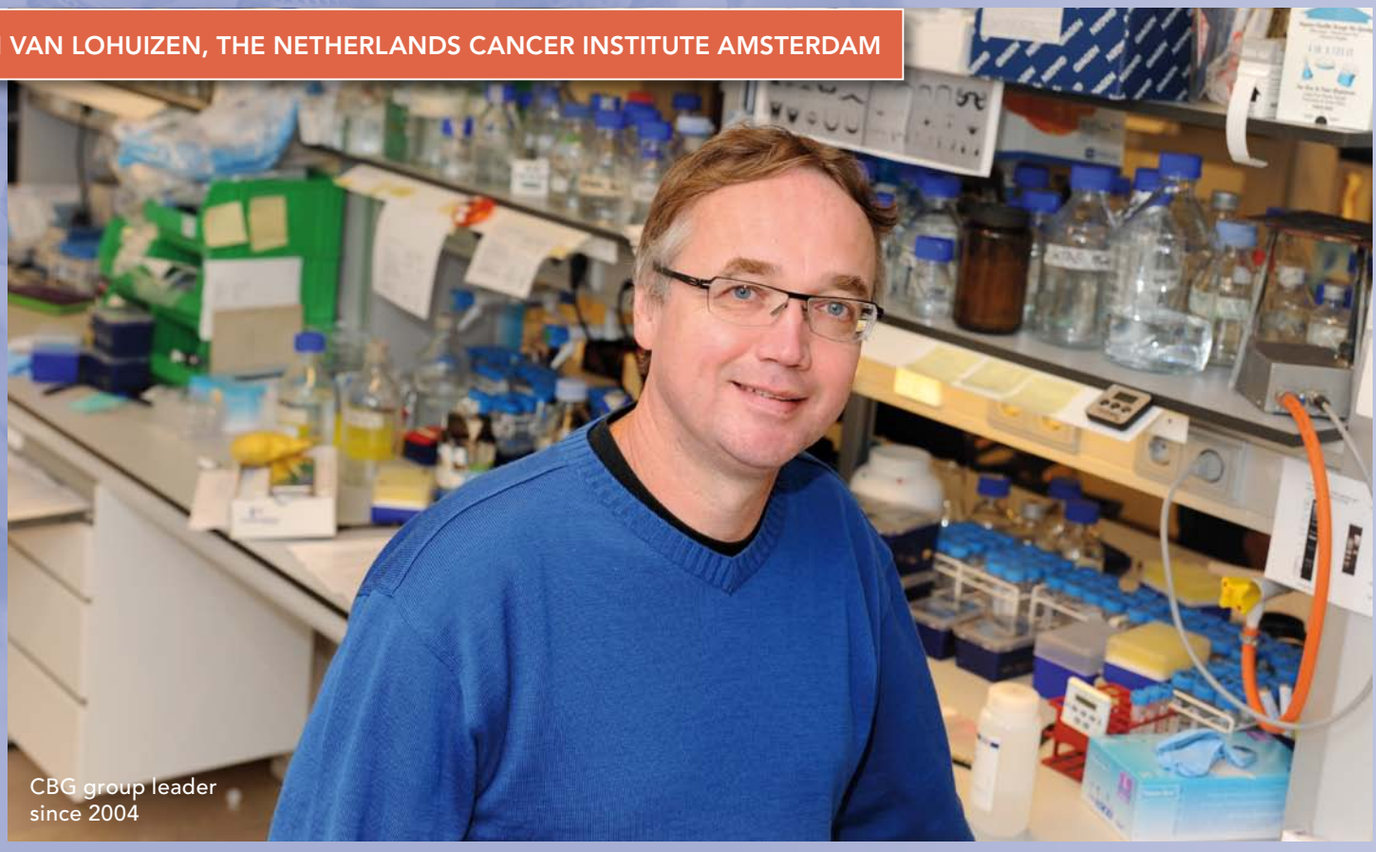
'With our research we add more 'cogs' to the machine of biology. Being a member of the CBG ensures that these cogs are applied swiftly and smoothly into the research themes of the other members of the CBG.'

the repressor that induce highly specific binding with the base pairs of the operator. These data complete our view of how transcriptional regulation by means of the *lac* repressor works.'

Pinpointing domains in DNA repair proteins

Other recent research by Kaptein's group focussed on the human ERCC1–XPF complex. Kaptein explains, 'This was a typical CBG project – a line of research initiated by Jan Hoeijmakers who is studying DNA repair mechanisms. One of these mechanisms is the nucleotide excision repair (NER) process, which is able to remove a broad range of DNA damages. Nucleotide excision repair protects organisms against DNA damage-induced carcinogenesis and premature aging. Its significance is illustrated by the severe clinical consequences of inherited defects in the system – they give rise to various syndromes, including the cancer-prone and photohypersensitivity disease xeroderma pigmentosum, the premature aging Cockayne syndrome, and the brittle hair disorder trichothiodystrophy. This repair mechanism depends on the presence several proteins that must act and interact at exactly the right place at the right time. To fully comprehend it, all the molecular participants in the process must be identified. It is also necessary to understand the three-dimensional structure of these participants and the domains that are involved in their actions and interactions. Therefore we recently analysed the human heterodimeric endonuclease complex known as the ERCC1–XPF complex. This complex performs an essential late step in the nucleotide excision repair process. It nicks, together with XPG (another nuclease) the damaged DNA strand at the 5' side of a helix-distorting lesion. In addition, the complex is involved in the recombinational repair of interstrand cross-links. By analysing both protein–protein interactions and protein–DNA interactions with NMR spectroscopy we were able to elucidate the three-dimensional structure of the complex's interaction domains. Our data show that ERCC1 is primarily responsible for binding to damaged DNA and XPF brings about the cleavage of DNA. We cannot foresee just how important this information on nucleotide excision repair will prove to be for other scientists, but it is one more cog in the machine.'

MAARTEN VAN LOHUIZEN, THE NETHERLANDS CANCER INSTITUTE AMSTERDAM



CBG group leader since 2004

A major switch in cell differentiation

It might be a real mouthful, but the title of the AMC-professorship awarded to Maarten van Lohuizen describes *exactly* what his profession is – Biology and Epigenetic Regulation of Normal and Cancer Stem Cells. Epigenetics is the study of the regulation of gene expression that is not based on changes of genetic material but on changes in the structure of the chromatin – the spatial structure of the genome. It intrigues Van Lohuizen how those epigenetic changes determine the difference between a normal stem cell, a cancer stem cell and a normal differentiated tissue cell. The Polycomb protein complexes in cells are the major focus of his studies.

How do stem cells such as embryonic stem cells differentiate into somatic cells such as astrocytes or neurons in the brain? Protein complexes of the Polycomb group play a crucial role in the differentiation, development and proliferation of cells, discovered Van Lohuizen. These protein complexes stick to chromatin at selected spots and prevent the expression of particular genes. 'Polycomb activity is a main switch in the determination of stem cells to stay as stem cells or to start a program of differentiation into other cell types. But if there is an aberrant abundance of one of the Polycomb proteins, the cells can become cancer cells. Polycomb proteins can silence several tumour- suppressor genes. In virtually all cancers we do see a deregulation of Polycomb activity and reverse differentiation in the direction of stem cells. If there is too much Polycomb protein, differentiation into somatic cells will be prevented and the cells will stay as stem cells for longer – and this is a risk for developing cancer.'

CV

Maarten van Lohuizen heads the Division of Molecular Genetics, NKI-AvL and is professor at University of Amsterdam Medical School. After a postdoctoral period (UCSF, I. Herskowitz) he has been a group leader at The Netherlands Cancer Institute (NKI-AvL), Amsterdam (from 1995). His group has made important contributions on the functional analysis of epigenetic gene silencing mechanisms by Polycomb-group (PcG) protein complexes, which play crucial roles in controlling development, differentiation and cell proliferation and when deregulated contribute to cancer formation. His group has also developed genome wide genetic screens in cell-based assays and in cancer-prone mice to identify new genes that contribute to cancer and classify them in functional groups/signaling pathways. In addition, his group has demonstrated a crucial role for Bmi1/PcG protein complexes in maintaining hemapoietic, neuronal, epithelial and embryonal stem cell fate. Unraveling the role of Bmi1/PcG in stem cell fate versus differentiation decisions and the consequence of this for cancer (stem) cell biology is a major focus of his group.

Goals

To study the mechanism of stable inherited transcriptional repression by Polycomb-group (Pc-G) protein complexes, and the effects of deregulation of Pc-G genes on development, cell cycle control, cancer formation and stem cell maintenance. A second main goal is to improve performing large-scale genetic screens in primary cells and in cancer-predisposed mice to identify cancer-relevant networks of collaborating oncogenes and tumour-suppressor genes.

Facilities and expertise

Epigenetic silencing mechanisms; Chromatin profiling, 4-C stem cell biology; Developmental regulation by Polycomb silencers; Conditional mouse models for cancer involving Polycomb-group genes; Large-scale genetic screens for cooperating oncogenes & tumour suppressor genes; High-throughput *in vivo* transposon & retroviral mutagenesis; Primary cell culture, ES and iPS culture; Retroviral & lentiviral transduction (siRNA & cDNA libraries).

Polycomb proteins, methylation and cancer

A major question that Van Lohuizen seeks to answer is whether it is possible to diminish the activity of the Polycomb enzymes and consequently prevent cancer. Together with pharmaceutical companies he searches for drugs with such an ability. Apart from their research in fruit flies (in which the Polycomb proteins were originally discovered) Van Lohuizen's group at the division of Molecular Genetics at the Netherlands Cancer Institute (NKI) studies the proteins in cell cultures and in mouse models. 'Genetically altered mice have been made at the NKI. These animals have mutations in the important tumour-suppressor genes *Brca1* and *p53* that causes breast cancer in humans. We see also very high cellular levels of Polycomb proteins in the tumours of these animals. Tumour cells derived from these mice appear very sensitive for the effect of inhibitors of Polycomb. We use those mouse models for a better understanding of the mechanism behind the Polycomb proteins and to search for small molecules that apply the brakes on Polycomb.'

> 'It is fascinating to see the enzyme "at work". It is also intriguing because the process is so fundamental to the distinction between stem cells and somatic cells.'

The principal function of the Polycomb enzymes seems to be the methylation of histones. These proteins are involved in maintaining chromatin structure and regulation of gene expression. A small molecule capable of blocking that methylation could also block the tumourigenic properties of Polycomb. But Van Lohuizen fears there will probably not be one simple solution, because the group of Polycomb proteins is very complex. In fact, there are several different Polycomb complexes. One of the major complexes consists of five proteins, and the other of fourteen proteins (in different forms). The five-protein complex binds to histone and attaches three methyl-groups to the protein. The fourteen-protein complex binds thereafter and sets a so-called 'ubiquitin flag' on the histones, which puts a real lock on the expression of the genes.

MAARTEN VAN LOHUIZEN, THE NETHERLANDS CANCER INSTITUTE AMSTERDAM

Key Publications

1 Jacobs JJL, Scheijen B, et al (1999). Bmi-1 collaborates with c-Myc in tumorigenesis by inhibiting c-Myc-induced apoptosis via INK4a/ARF. Genes Dev. ; 13: 2678-90.

2 Jacobs JJL, Kieboom K, et al (1999). The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the INK4a locus. Nature ; 397: 164-8.

3 Jacobs JJL, Keblusek P, et al (2000). Senescence bypass screen identifies TBX2, which represses Cdkn2A (p19ARF) and is amplified in a subset of human breast cancers. Nature Genet. ; 26: 291-99.

4 Lund AH, Turner G, et al (2002). Genome-wide retroviral insertional tagging of genes involved in cancer in Cdkn2a-deficient mice. Nature Genet. ; 32: 160-65.

5 Leung C, Lingbeek M, et al (2004). Bmi1 is essential for cerebellar development and is overexpressed in human medulloblastomas. Nature 2004; 428: 337-41.

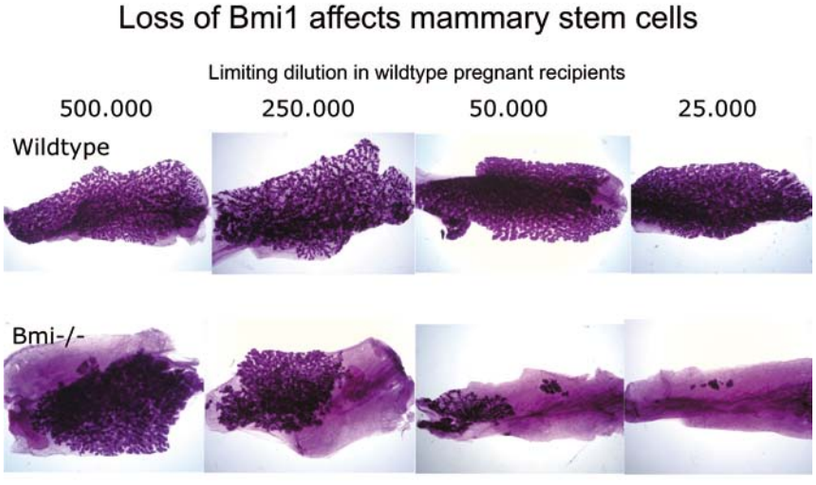
6 Bruggeman SWM, Valk-Lingbeek ME, et al (2005). Ink4a and Arf differentially affect cell proliferation and neural stem cell self-renewal in Bmi1 deficient mice. Genes Dev.; 19: 1438-43.

7 Tolhuis B, de wit E, et al (2006). Genome-wide profiling of PRC1 and PRC2 Polycomb chromatin binding in Drosophila. Nature Genet. ; 38: 694-9.

8 Bruggeman SWM, Hulsman D, et al (2007). Bmi1 controls tumor development in an Ink4a/Arf-independent manner in a mouse model for glioma. Cancer Cell; 12: 328-41.

9 Uren A.G, Kool J., et al (2008). Large scale mutagenesis in p19ARF and p53 deficient mice identifies cancer genes and their collaborative networks. Cell; 133, 727-41.

10 Pietersen AM, Evers B, et al (2008). Bmi1 Regulates Stem Cells and Proliferation and Differentiation of Committed Cells in Mammary Epithelium. Curr Biol.;18:1094-9.



Mammary fat pad transplantation assay measuring stem cell activity.

When limiting amounts of ductal cells are transplanted, failure of outgrowth indicates a severe stem cell defect in mice deficient for the Polycomb group gene Bmi1 (bottom row) when compared with wild type mice (top row).

> Van Lohuizen: 'We have the strong impression that Polycomb also influences the tertiary structure – the complex three-dimensional structure – of chromatin.'

Van Lohuizen's research gave birth to the detection of several Polycomb proteins and their connection to cancer genes. He also elucidated, in cooperation with NKI crystallographer Titia Sixma, the structures and mechanisms of some of the Polycomb proteins. 'It is fascinating', Van Lohuizen says, 'to see the enzyme "at work". It is also intriguing because the process is so fundamental to the distinction between stem cells and somatic cells. It provokes the question: What makes a stem cell? Maybe we can use our discoveries to develop a new complementary therapy for cancer, but my primary incentive is to know more about how cells differentiate. It is amazing that the new techniques for screening the genome are so effective and lead to more and more new genes involved in the Polycomb system. The funding from the CBG is an important force behind developments in this field. Several of my postdocs and students have benefited well from it. In the Netherlands nowadays it is increasingly difficult to engage in riskier research topics and you often have to know your results in advance to benefit from most financial resources for research. I fear that

Current group members

Research Associate
Anna Pavlina Haramis

Postdocs
Elisabetta Citterio (senior)
Jean Bernard Beaudry
Gaetano Gargiulo
Karim Nacerddine
Nienke de Vries
Anke Sparmann
Bas Tolhuis
Bart Westerman
Cesare Lancini
Inka Pawlitzky

PhD students
Martijn Koppens
Joep Vissers
Yme van der Velden

Technicians
Els Verhoeven
Ellen Tanger
Danielle Hulsman
Paulien Cornelissen
Marleen Blom

requirement puts the lid on the advancement of science and – in the end – on the development of new medical therapies, including those for cancer.'

The value of screening for detecting cancer genes

Van Lohuizen and his colleagues have profited from the CBG's investment in their large-scale screening facility and the large-scale use of insertion mutagenesis with retroviruses. These viruses tend to incorporate into DNA like a transposon. When they hit a gene – mostly accidentally – that gene is either knocked out or becomes activated, depending on precisely where they insert into the DNA. Sequencing the DNA fragments on both sides of the transposons resulted in the detection of a lot of genes that were involved in cancer. Van Lohuizen explains: 'We selected the cells on their cancer abilities. Therefore we know that the detected hits were in genes that were responsible for cancer. Using mice, we found 600 tumours with about 10,000 insertions – a mean of 15 to 20 hits per tumour. Only about 330 genes were responsible for the development of cancer. Some of those were very often found damaged in the tumours. The interesting thing is that not only were the genes detected, but also micro-RNAs and regulating (and highly conserved) elements. They can be structural elements or DNA binding sites. Those broad screens yielded new candidates for genes on the pathway for cancer. Analysing the patterns in which different genes are found together or excluded in tumours tells us something about the networks of proteins involved in the origin of cancer. The advantage of this simultaneous approach (compared with the sequencing of a cancer genome) is that it gives us the chance of recognising the relation between cause and effect. Solely detecting mutations in a cancer genome cannot specifically determine whether that mutation is causal or coincidental.' Van Lohuizen and his co-workers carried out their experiments in genetically altered mice that were prone to cancer. Their experiments can now easily be compared with cultures in human tumour tissue, by using comparative genome hybridisation techniques.

WOUTER MOOLENAAR, THE NETHERLANDS CANCER INSTITUTE AMSTERDAM

CV

Wouter Moolenaar studied biophysics at the University of Groningen and obtained his PhD degree (cum laude) in 1978 at Leiden University on a thesis on neuroblastoma cell electrophysiology. He spent postdoctoral periods at the Weizmann Institute of Science (Rehovot, Israel) in the groups of dr. U. Littauer and dr. J. Schlessinger to work on neuronal excitability and growth factor signaling, respectively. As from 1980 he continued his work on growth factor signaling at the Hubrecht Laboratory in Utrecht. In 1988 he became group leader at the Netherlands Cancer Institute in Amsterdam, where he discovered and characterized LPA as a novel lipid growth factor acting through G protein-coupled receptors. In 1991 he was elected as EMBO member and in 1997 appointed professor (part-time) of Molecular Cell Biology at Leiden University.

The role of lipids in carcinogenesis

Not laughing out loud, perhaps, but wry smiles could be seen on the faces of fellow scientists at the Netherlands Cancer Institute (NKI) when Wouter Moolenaar first espoused the importance of a phospholipid as a growth factor in the development of cancer. Genes and proteins … of course! But lipids? No way! From the 1980s, as group leader of NKI's Division of Cell Biology, Moolenaar has supported the role of his 'baby', lysophosphatidic acid (LPA), as a growth factor for many cell types. LPA is in its adolescence and is now fully accepted in the scientific world of cancer research.

Extending his thanks to CBG, Moolenaar adds: 'It is very uncommon for lipids to act as growth factors – they are mostly peptides. But my group discovered LPA was a growth factor in the late 1980s. To postulate that lipids are involved in the regulation of cell proliferation is no longer considered strange. Now there are six or seven known cell-surface receptors for LPA, all of which involved in important processes like cell proliferation, motility and survival. Although we don't understand the function of half of them', Moolenaar confesses, 'it is difficult to take a new research direction and approach a risky series of studies in which there are no results yet. For achieving that goal, the CBG grant was perfect. It was more or less ''free'' money, with very easy accountability, and this approach has certainly borne fruit.'

A lipids as a growth factor – a reality

The lipid growth factor is now common in the

CBG group leader since 1998

Goals

To establish the importance of lysophospatidic acid (LPA) as a lipid growth factor in health and disease, particularly its role in tumour progression. In addition to characterizing LPA receptor signaling pathways, the focus is on the role of the major LPA-producing enzyme, termed autotaxin, during development and in adult life.

Facilities and expertise

State-of-the-art cell biological techniques, live-cell imaging, gene expression profiling and knockout mouse studies. Collaboration with chemists and structural biologists at the NKI to characterize small-molecule inhibitors of autotaxin and to solve its crystal structure.

scientific literature. One receptor Moolenaar characterised belongs to the so-called G-protein-coupled receptor family and is highly specific for LPA. Binding of LPA to this receptor stimulates cell proliferation and motility – both important for the metastatic properties of cancer cells. In addition, LPA evokes profound changes in the cell cytoskeleton, altering it from what is usually a well-spread, flattened shape to a rounded one; such rounded cells readily detach from their neighbours and the extracellular matrix. 'So with this LPA–receptor interaction and its downstream signals, we are working at the front line of the cancer problem. We would still like to explore the importance of LPA signalling for tumour maintenance and whether it is possible to inhibit LPA's action.'

'With this LPA–receptor interaction and its downstream signals, we are working at the front line of the cancer problem.'

LPA and the autotaxin enzyme ATX

The lysolipid is normally bound to albumin and flows in the bloodstream at low concentrations. Several tissues and cell types make LPA locally, and secrete an enzyme that produces the lipid outside the cell. One specific lysophospholipase D, named autotaxin (ATX), is secreted from the cell interior into the extracellular environment. It is involved in the local production of LPA from the precursor, lysophosphatidylcholine (LPC). Moolenaar explains: 'The production of ATX is crucial to the formation of LPA. Indeed, it is the limiting factor. It leaves the cell in the normal way by vesicular transport, and in some types of human cancer the amount of ATX in the cells is upregulated. LPA stimulates cell growth and cell motility, so it is important to study ways of inhibiting it, for instance by using small molecules that inhibit the enzymatic activity of ATX.'

In recent years, Moolenaar has collaborated with Tassos Perrakis's structural biology group at the NKI to shed light on the spatial structure of ATX. They now can visualise the three-dimensional

WOUTER MOOLENAAR, THE NETHERLANDS CANCER INSTITUTE AMSTERDAM

Key publications

1 Postma F, Jalink K, et al (2001). Ga(13) mediates activation of a depolarizing chloride current that accompanies RhoA activation. Curr Biol. 11:121-4.

2 Van Leeuwen FN, Olivo C, et al (2003). Rac activation by LPA1 receptors through the guanine nucleotide exchange factor Tiam1. J Biol Chem. 278:400-6.

3 Mills GB, Moolenaar WH (2003). The emerging role of LPA in cancer. Nat Rev Cancer 3:582-91.

4 Van Meeteren LA, Frederiks F, et al (2004). Spider and bacterial sphingomyelinases D target cellular LPA receptors by hydrolyzing lysophosphatidylcholine. J Biol Chem. 279:10833-6.

5 Van Meeteren LA, Ruurs P, et al (2005). Inhibition of autotaxin by LPA and sphingosine 1-phosphate. J Biol Chem. 280:21155-61.

6 Van Meeteren LA, Ruurs P, et al (2006). Autotaxin, a secreted lysophospholipase D, is essential for blood vessel formation during development. Mol Cell Biol. 26:5015-22.

7 Van Meeteren LA, Moolenaar WH (2007). Regulation and biological activities of the autotaxin-LPA axis. Prog Lipid Res. 46:145-60.

8 Van Zeijl L, Ponsioen B, et al (2007). Regulation of connexin43 gap junctional communication by phosphatidylinositol 4,5-bisphosphate.J Cell Biol. 177:881-91; 2007.

9 Stortelers C, Kerkhoven R, Moolenaar WH (2008). Multiple actions of LPA on fibroblasts revealed by transcriptional profiling. BMC Genomics 9:387.

10 Ponsioen B, van Zeijl L, et al (2009). Spatiotemporal regulation of chloride intracellular channel protein CLIC4 by RhoA. Mol Biol Cell. 20:4664-72.

structure of the active site of the enzyme and they can optimise the properties of the small molecules that fit the best in that active environment. Moolenaar and his collaborators also discovered that knocking out the ATX gene in mice leads to the death of embryos in utero – ATX is therefore crucial for the development in the embryonic stage. Moolenaar says: 'Those embryos fail to make blood vessels. The vascular system is one of the first organs to be made in embryogenesis. We are now trying to switch off the ATX gene in adult mice, but there is still a long way to go.'

As a cell biologist, Moolenaar is also interested in the function of LPA at the molecular level. How is the growth signal transduced from the cell surface into the cell interior? He continues: 'Colleagues of mine have shown that mice with colon cancer over-express the ATX enzyme and one particular LPA-receptor. Disabling that LPA receptor protects them from progression of the cancer. Conversely, forced over-expression of ATX or individual LPA receptors in mouse mammary glands leads to mammary carcinoma. These results are very interesting but it remains to be seen whether they hold for human cancers of the colon or breast. Right now there are several pharmaceutical companies who are looking for small molecules to inhibit specific LPA-receptors, with the hope of reducing development of tumour cells.' Together with chemists at the NKI, Moolenaar has patented some small molecules that inhibit ATX and thereby prevent the production of LPA. He adds: 'Chemists in the industry have selected the most promising of those leads and are proceeding to transform them into a feasible drug'.

Potential for new anticancer drugs

Moolenaar is proud that after all the years he's invested in this lipid, his approach has yielded not only scientific success (he was the first to discover LPA and elucidate its signalling properties) but also some interesting leads for drug discovery. 'I was lucky to become a partner in the CBG and the CBG grant made it possible for me to pursue the path of ATX.' But there are still more, huge challenges to overcome in this field of research. There is still much to learn about the function of different LPA receptors. 'In fact, they all work in

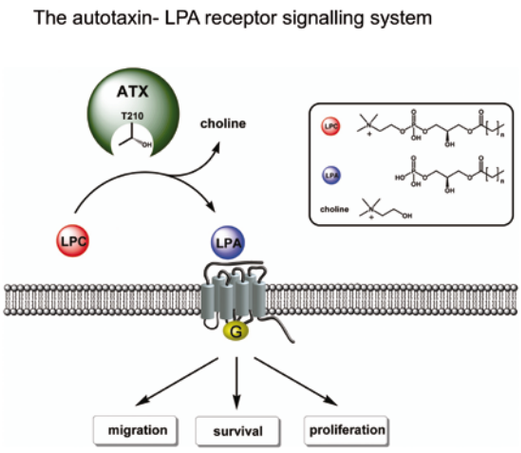
Current group members

Staff
Kees Jalink

Postdocs
Elisabetta Argenzio
Bas Ponsioen
Maikel Jongsma
Leonie Van Zeijl

PhD students
Harald Albers
Dalila Elouarrat
Anna Houben

'Right now there are several pharmaceutical companies who are looking for small molecules to inhibit specific LPA-receptors, with the hope of reducing development of tumour cells.'



The autotaxin-LPA receptor signalling system.

Autotaxin (ATX) is a secreted enzyme that produces the lipid growth factor lysophosphatidic acid (LPA) from extracellular lysophosphatidylcholine (LPC). Newly produced LPA acts on specific G protein-coupled receptors in the plasma membrane of many cell types, resulting in enhanced cell proliferation, migration and survival.

the same manner', Moolenaar states, 'but there is overlap in the pathways along which the signals are transported into the cell. Some of the receptors direct a different process. For example, usually the LPA receptor stimulates cell proliferation and alters the cytoskeleton, facilitating cell migration and metastasis. But there is at least one LPA-receptor that actually inhibits cell migration while stimulating cell growth. The significance of that should be investigated.'

What's more, LPA does not stand alone. There is another phospholipid – a sphingolipid – with the same kind of receptors and signalling properties as LPA. This one is typically involved in immune responses (notably lymphocyte trafficking and inflammation), and therefore Moolenaar expects that bioactive lipids like LPA not only play a role in tumour progression but also in other disease processes, such as chronic inflammation.

JACQUES NEEFJES, THE NETHERLANDS CANCER INSTITUTE AMSTERDAM

CV

Jacques Neefjes studied chemistry and moved into the fields of cell biology and immunology during his PhD training period. He did a post-doc in Heidelberg before starting his group at the Netherlands Cancer Institute in 1994. He initially studied the cell biology of MHC class I and II presentation and defined many steps in these pathways. He defined the receptor for dynein motor binding to late endosomes which at time was the first receptor understood. He later determined how cholesterol is controlling this motor by unique interactions between the ER and late endosomes. Neefjes was one of the first groups using GFP in Cell Biology and developed many tools for 'single cell biochemistry' on living cells. More recently, Neefjes merged these fields with chemical biology and developed and applied techniques to define target-lead combinations for various processes. Neefjes received the Golden Medal of the Chemical Society for his work. He is EMBO member, Head of the Division of Cell Biology at the NKI and professor at the University of Leiden.

Networks across the disciplines

Jacques Neefjes, working at the NKI, likes networks. He studies them concerning the subject of his own research, but also looks for connections with areas outside that scope. Neefjes has changed his policy from studying individual genes and single proteins to investigating networks of genes and proteins. At the same time he involves different scientific disciplines in his research. With his own studies on the immunology of tumour defences, Neefjes expects to gain the best results by combining chemistry with the cell biology of the immune response. Such a combination should yield knowledge of biochemical pathways and chemical leads that enable manipulation of such pathways and thereby the immune response.

Trained as a chemist, Neefjes finds himself on the borderline of immunology, cell biology and chemistry. His goal is to use cell biology to understand the responses of the immune system and to use chemistry to identify the compounds that are able to manipulate the immune system. Such goals require a serious investment in state-of-the-art technology. 'If a Dutch researcher wants to be able to participate in the scientific rat race, he or she will have to invest in the latest technology and dedicated staff, for instance, in high-throughput systems that are able to gather large quantities of information. Only these things will lead to new research and papers in top journals.' That is why Neefjes supports the decision of spending a significant part of the CBG budget on sophisticated equipment.

Neefjes is the head of the division of Cell Biology of the Netherlands Cancer Institute (NKI). He aims at identifying so-called target-lead combina-

CBG group leader since 2008

Goals

To combine chemical biology with cell biology and biochemistry using high throughput tools such as siRNA and chemical library screening. To understand how to manipulate the immune system.

Facilities and expertise

Facilities: micro-injection, confocal microscopy.
Expertise: single cell biology tools; image analysis; chemical biology.

tions. These are small chemical compounds that have the ability to inhibit the activity of proteins, and therefore have biological activity. This parallels the effect of small nuclear RNAs (snRNAs) that down-regulate the expression of selected genes. The processes of chemically inhibiting an enzyme and removing an enzyme from a cell are similar and should give similar effects. 'Inhibiting functions in a chemical and a genetic way at the same time is something that not many researchers are doing yet', Neefjes says. 'We are interested in the off-target properties of existing drugs that may give the side effects of medication. If one finds a favourable side effect, perhaps it can be modified away from its 'official effect' and biased towards the desired side effect. This can evolve into a therapy, for instance against cell growth or metastasis, or to stimulate the immune system to stop cancer cells from doing harm.' Neefjes' group has the equipment to make a genome-wide analysis of the effect of different drugs on certain properties of cells. Neefjes says 'It takes three years to do so and these are high-risk projects, typical of academic research. That is why the pharmaceutical industry will not easily do such projects.'

'We are actually working on solving a very complex puzzle about how protein networks act in cells.'

Exploring the immune response to tumours

Neefjes studies a problem of the immune system when fighting tumour cells: creating a strong defence using antibodies after a vaccination and better immune therapy using T cells. However, this defence response cannot be too strong because it will yield autoimmune responses. Neefjes and his colleagues focus on the major histocompatability complex (MHC) system. MHC molecules present fragments of other proteins at the cell surface to the immune system. MHC class I molecules present fragments from within the cell to give a 'kill me' signal. MHC class II molecules present fragments from cells and from pathogens to helper T cells. This is essential to generate

JACQUES NEEFJES, THE NETHERLANDS CANCER INSTITUTE AMSTERDAM

Key publications

1 Reits,E.A., Vos,J.C., et al (2000). The major substrates for TAP in vivo are derived from newly synthesized proteins. Nature 404, 774-778.

2 Jordens,I., Fernandez-Borja,M., et al (2001). The Rab7 effector protein RILP controls lysosomal transport by inducing the recruitment of dynein-dynactin motors. Curr Biol 11, 1680-1685.

3 Reits,E., Griekspoor,A., et al (2003). Peptide diffusion, protection, and degradation in nuclear and cytoplasmic compartments before antigen presentation by MHC class I. Immunity 18, 97-108.

4 Michalides,R., Griekspoor,A., et al (2004). Tamoxifen resistance by a conformational arrest of the estrogen receptor alpha after PKA activation in breast cancer. Cancer Cell 5, 597-605.

5 Reits,E., Neijssen,J., et al (2004). A major role for TPPII in trimming proteasomal degradation products for MHC class I antigen presentation. Immunity 20, 495-506.

6 Neijssen,J., Herberts,C., et al (2005). Cross-presentation by intercellular peptide transfer through gap junctions. Nature 434, 83-88.

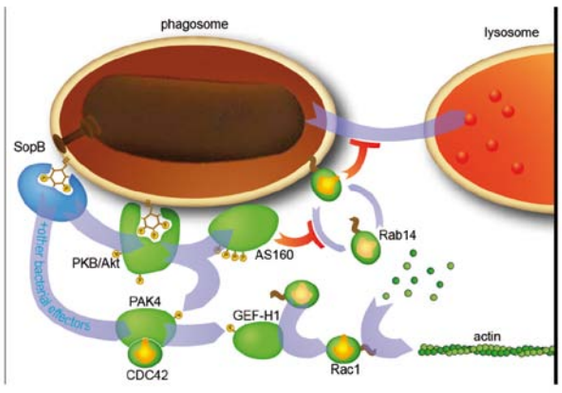
7 Zwart,W., Griekspoor,A., et al (2005). Spatial Separation of HLA-DM/HLA-DR Interactions within MIIC and Phagosome-Induced Immune Escape. Immunity 22, 221-233.

8 Reits, E.A., Hodge, J.W., et al (2006). Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. J. Exp. Med. 203, 1259-71.

9 Kuijl,C., Savage,N.D., et al (2007). Intracellular bacterial growth is controlled by a kinase network around PKB/AKT1. Nature 450, 725-730.

10 Rocha N, Kuijl C, et al (2009). Cholesterol sensor ORP1L contacts the ER protein VAP to control Rab7-RILP-p150 Glued and late endosome positioning. J Cell Biol.185 :1209-1225.

The model for AS160/Rab14 and PAK4



An siRNA and chemical screen revealed kinases acting in a complicated cell biological manner to control intracellular survival of bacteria. Salmonella is secreting a factor in the host cytosol called SopB, This is activating PKB/Akt1 which then activates the kinase PAK4 that controls actin polymerization. In addition, PKB/Akt1 phosphorylates AS160 that then becomes inactivated. Since AS160 is the GAP for the GTPase Rab14, Rab14 becomes activated and prevents transfer of phagosomes to lysosomes. This cell biology explains how bacteria survive inside cells and why PKB/Akt1 inhibitors eliminate intracellular bacteria.

antibodies and a proper immune response to tumours. 'Can we manipulate this MHC-II system by silencing selectively the expression of every individual protein in a cell using siRNA libraries?', Neefjes wondered. 'To perform such a huge task, we needed combinations of techniques that I did not know about three or four years ago. For instance: robotic steps, advanced microscopy techniques, quantitative PCR (polymerase chain reaction) and large-scale data analysis.'

This exercise yielded many new proteins and networks of proteins controlling MHC-II molecules. However, for genuine manipulation, chemical compounds are required. So Neefjes performed a comparable exercise using a database consisting of 2,000 chemicals, including all the presently used medicines. He compared the results from these assays to those of his earlier siRNA screen to find target proteins and the compounds targeting them. 'After screening the cells using the siRNA database, we found 280 proteins that are involved in regulating the MHC class II. This is far too many proteins to study in detail, so this makes it complicated to place each protein in a certain pathway.'

Current group members

Postdocs
Coenraad Kuijl
Victoria Menendez
Tiziana Scanu
Charlotte Sadaka
Gosia Garstka

PhD students
Izhar Salamon
Petra Paul
Tineke van den Hoorn,
Marlieke Jongsma
Baoxu Pang
Sjoerd van Deventer
Ruud Wijdeven

Technician
Lennert Janssen

'If a Dutch researcher wants to be able to participate in the scientific rat race, he or she will have to invest in the latest technology and dedicated staff.'

Screening of cluster proteins and networks

For this reason Neefjes developed secondary screens to cluster proteins with similar effects on cells by the different siRNAs. 'We have used a lot of computing power and programming in the process, some of which were self-made, and some which were put at our disposal by others. By using the newest analytical techniques, the data files start to bear fruit. What is fascinating about all this is that we are actually working on solving a very complex puzzle about how protein networks act in cells. It cannot be solved by studying single proteins without the context of other proteins acting in the same cluster. That is why we study networks of proteins. It is much faster and better at describing the real biology, even though it is still extremely complicated.'

'At first sight there doesn't seem to be an obvious link between the immune system and cancer,' Neefjes acknowledges. 'And it is not that I think of cancer every second while doing my research, but if one can stimulate MHC class II and the immune system, this can lead to an immune responses against tumour cells. If the tools and targets for manipulating the immune system are found, there will be chemicals and antibodies to focus on these targets. And we have currently about 50,000 different compounds at our disposal. These include compound libraries, made by colleagues, that target enzyme classes not yet tested for by the pharmaceutical industry.'

The search for combinations of leads and targets can occur within a scientific group such as Neefjes' group. The next step – medical chemistry – in which the lead is improved and adjusted to make sure it is not broken down by the liver and has optimal pharmacokinetic properties is the task of the industry. Neefjes adds: 'We are working together with Organon, now called MSD, in Oss. They finance part of our high-throughput screening activities. In exchange, the researchers of MSD are kept up-to-date with our findings and they are allowed to look at our data before we publish it in a scientific journal. This is a beneficial collaboration for both sides that helps us improve our fundamental research, which may also make application of the results more realistic.'

GERTJAN VAN OMMEN, LEIDEN UNIVERSITY MEDICAL CENTER

CBG group leader
since 1998

The freedom to be the first

An innovative approach to science and technology has brought GertJan van Ommen and his group several breakthroughs in different areas of human genetics and genomics. Among them are a revolutionary therapeutic approach to Duchenne's muscular dystrophy and the first genomic sequence of a named female human. 'CBG provides a relatively small part of our budget, but it gives us the freedom to act when we see an opportunity, without the usual chore of applying for a grant or needing to convincing our paymasters. Thanks to CBG, we sometimes get to be the *first* to do something.'

Being *doubted* may never become commonplace for Van Ommen, a Leiden professor of Human Genetics, but it can be the consequence of being ahead of one's time. Van Ommen remembers: 'When, in 1995, we first proposed exon skipping as a potential way to cure patients with Duchenne's muscular dystrophy, the field laughed at the idea. When we asked our institute for a next generation DNA sequencer in 2006, the board turned it down after colleagues questioned the value of it and said it would be standing idle most of the time. Thanks to CBG, we could pursue those plans.'

'Now the clinical trials show that exon skipping may very well work in human patients and GlaxoSmithKline has pledged to invest about half a billion euros in Prosensa, the company that put exon skipping on the market. The new generation sequencer we installed with CBG money, the first of its kind on the European mainland, has been busy around the clock

CV

GertJan van Ommen, PhD, is head of the Department of Human Genetics of Leiden University Medical Center (LUMC), and founder of the Leiden Genome Technology Center (LGTC), a principal genomics facility in the Netherlands. He directs the NGI-funded Center for Medical Systems Biology CMSB, and coordinates the Dutch biobank infrastructure BBMRI-NL. He is treasurer of P3G, board member of the Medical Genetics Centre (MGC), past president of HUGO and the European and Dutch Societies of Human Genetics, and Editor-in-chief of the European Journal of Human Genetics. He has as major research interests neuromuscular and neurodegenerative diseases, with a focus on Duchenne muscular dystrophy (DMD) and Huntington Disease; and the development of genome technology for disease study, diagnosis, therapy and prevention, and societal aspects of genetics. He participates in several EU FP7 Projects including BBMRI, the European biobanking infrastructure, and ENGAGE. As of 1998, his group has been pioneering the exon-skipping approach for therapy of DMD, the therapy now closest to market. Coordinated by the Leiden University spinoff company Prosensa, in 2006 the first-in-man study was performed and in 2009 the first successful phase I/II systemic trial.

Goals

To apply innovative approaches to the unraveling of causes of genetic disease, with an emphasis on neurological and neuromuscular disease. The ultimate aim is the development or improvement of diagnostics, prognostics, therapy and prevention.

Facilities and expertise

Expertise is in high-throughput genomics, arraying and deep sequencing, the application of transcriptomics and proteomics biomarker development to characterise disease states and monitor therapeutic success; the application of exon skipping to modulate gene expression and function, and the development of Llama intrabodies to intervene with protein toxicity.

within half a year – so we've had to apply for another one!'

Hope for Duchenne children

Arguable one of the most inspiring translational results from the CBG community is the therapy for Duchenne's muscular dystrophy (DMD), a sex-linked hereditary disorder. DMD is caused by a mutation in the dystrophin gene, the largest known gene in the human genome – and it is probably too large for a classical gene therapy approach. The Leiden group found that the mutations in DMD patients always involve a reading frame shift, causing an early termination of dystrophin synthesis. If a mutation does not cause a reading frame shift, the patient has much milder hereditary disease known as Becker's muscular dystrophy (BMD).
Judith van Deutekom and others within Van Ommen's group developed a revolutionary method for using this knowledge in a genetic therapy called 'exon skipping'. The idea involves misleading the splicing apparatus of the cell with a complementary antisense oligonucleotide, so skipping an exon of the pre-mRNA in such a way that the reading frame is restored. The result is a truncated – but functional – dystrophin. Van Deutekom and co-workers succeeded in putting this idea to work, first in an animal model, and then in human patients. They showed a restoration of dystrophin production in human muscles after the injection of the oligonucleotide. And they proved the technique to be safe. Now clinical trials are underway in the biotech company called Prosensa. Van Ommen says: 'When you work on a disorder for so long, of course you hope you will find a cure. But we never expected it, especially not when we saw how huge the gene is – 2.4 million nucleotides! Now there is hope.'

'Clinical geneticist in training, Marjolein Kriek, was chosen as the first woman whose complete genome would be sequenced.'

Master switches

The Leiden Human Genetics team has been at the forefront of the genomics revolution from the

GERTJAN VAN OMMEN, LEIDEN UNIVERSITY MEDICAL CENTER

Key publications

1 Van Overveld PG, Lemmers RJ, et al (2003). Hypomethylation of D4Z4 in 4q-linked and non-4q-linked facioscapulohumeral muscular dystrophy. Nat Genet 35: 315-317.

2 Van Ommen GJB. (2005). Frequency of new copy number variation in humans. Nat Genet 37: 333-334.

3 Verheesen P, de Kluijver A, et al (2006). Prevention of oculopharyngeal muscular dystrophy-associated aggregation of nuclear polyA-binding protein with a single-domain ntracellular antibody.Hum Mol Genet. ;15(1):105-11.

4 't Hoen PA, van der Wees CG, et al (2006). Gene expression profiling to monitor therapeutic and adverse effects of antisense therapies for Duchenne muscular dystrophy. Pharmacogenomics. ;7(3):281-97.

5 Van Deutekom, J. C., A. A. Janson, et al (2007). Local dystrophin restoration with antisense oligonucleotide PRO051. N Engl J Med 357:2677-86.

6 Aartsma-Rus, A., A. A. Janson, G. J. van Ommen, and J. C. van Deutekom. (2007). Antisense-induced exon skipping for duplications in Duchenne muscular dystrophy. BMC Med Genet 8:43.

7 Van Roon-Mom, W. M., B. A. Pepers, et al (2008). Mutant huntingtin activates Nrf2-responsive genes and impairs dopamine synthesis in a PC12 model of Huntington's disease. BMC Mol Biol 9:84.

8 Alagaratnam, S., B. J. Mertens, et al (2008). Serum protein profiling in mice: identification of Factor XIIIa as a potential biomarker for muscular dystrophy. Proteomics 8:1552-63.

9 Pedotti, P., P. A. t Hoen, et al (2008). Can subtle changes in gene expression be consistently detected with different microarray platforms? BMC Genomics 9:124.

10 't Hoen PA, Ariyurek Y, et al (2008). Deep se -based expression analysis shows major advances in robustness, resolution and inter-lab portability over five microarray platforms. Nucleic Acids Res. ;36(21):e141. Epub 2008 Oct 15.

beginning. Van Ommen: 'I have been president of the Human Genome Organisation HUGO from 1998 to 2000. And until 2004 I was on its council as senior vice-president. As we established CBG, genomics was just gaining momentum. Now we are reaping the fruits of our work'. He and his colleagues always had a broad approach to genetics and genomics, from a medical perspective. While appreciating the work of scientists who focus on more basic questions concerning signalling and development, they concentrated on the applications of genetics and genomics in combating disease. This approach becomes clear at the Centre for Medical Systems Biology (CMSB) – one of the four Genomic Centres of Excellence of the Netherlands Genomics Initiative (NGI). Van Ommen established CMSB in 2003 together with colleagues from Leiden University, Erasmus MC in Rotterdam, Free University and Free University MC in Amsterdam and TNO Leiden. CMSB uses a systems approach to study the underlying mechanisms of common diseases like Alzheimer's disease, arthritis, depression, diabetes, migraine, immune disorders and cancer. These diseases, diverse as they may be in their outward appearance, have certain disruptions of the system in common. The researchers work from the hypothesis that these disruptions will lead them to 'biological master switches' in the human body, yielding multiple opportunities for therapy and prevention. In 2008, in the second round of funding of the NGI, the CMSB programme was continued, co-financed by the participating institutions.

After Watson – time for Kriek

With the advent of the next generation of DNA sequencers, Van Ommen saw new opportunities to expand knowledge about genomics. 'We have always been interested in innovative approaches. We were among the first to work with cosmids and YACs, we pioneered pulsed-field electrophoresis and came up with the first megabase map in 1986. In the 1990s, working with our colleagues from the cytogenetics department, we helped develop the use of multicolour fluorescent in situ hybridisation (FISH) to study the configuration of genes and chromosomes in nuclei. The same hybridisation method can also be applied to single-stranded DNA. This 'fibre FISH' is an excellent tool for

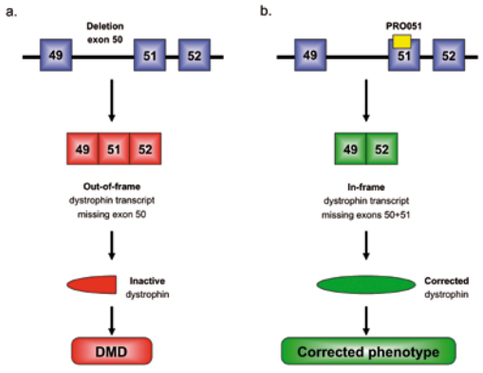
Current group members

Staff
Johan den Dunnen, Annemieke Aartsma, Willeke van Roon, Peter-Bram 't Hoen, Judith Boer, Barend Mons, Sjef Verbeek, Peter Devilee, Dorien Peters, Peter de Knijff, Silvère vd Maarel, Arn vd Maagdenberg, Ko Willems van Dijk

Senior scientists and postdocs
Jacopo Celli, Gerard Schaafsma, Jeroen Laros, Isabella Houweling-Gazzoli, Marcel Veltrop, Willem Hoogaars, Henk Buermans, Vishna Devi Nadarajah, Polina Reshetova, Peter Taschner, Ashwin Dihal, Marco Roos, Bharat Singh, Els Robanus Maandag, Maaike Vreeswijk, Vered Raz, Richard Lemmers, Rinse Klooster, Yvonne Krom, Jessica de Greef

PhD students
Jaap van der Heijden, Emmelien Aten, Yu-Ching Lai, Yu Sun, Hans Heemskerk, Maaike van Putten, Ingrid Verhaart, Melvin Evers, Anastasios Mastrokolias, Menno Schut, Matthew Hestand, Dwi Kemaladewi, Maarten van Iterson, Rezaul Karim, Herman van Haagen, Seda Yilmaz-Elis, Javier Martin Ramirez, Antoine de Morrée, Kim Rutgers, Peter Thijssen

Technicians
Sophie Greve, Yavuz Ariyurek, Rudy Pijpers, Arnoud Schmitz, Golan Vegter, Rolf Vossen, Michiel van Galen, Emile de Meijer, Paul Murphy, Ivo Fokkema, Michel Villerius, Laura van Vliet, Christa de Winter, Barry Pepers, Sandra van Heijningen, Margriet Hulsker, Silvana Jirka, Cor Breukel, Jill Claassens, Jos vd Kaa, Melissa Mulder



solving mapping problems in regions with many repeats. I expect that this method will come in handy in the near future, with loads of data emerging on DNA copy number variation between individuals, generated by chip approaches and next-generation sequencers'.

Thanks to the CBG, in 2006 Van Ommen ordered his first Solexa (now Illumina) next-generation sequencer. By the end of 2007, all the bureaucratic hoops had been jumped through and the machine could actually be installed. Contrary to the expectations of many people, it was soon running around the clock, sequencing DNA samples from many species and sources. To have enough capacity for their own research, the Leiden geneticists applied for a second sequencer. Meanwhile, in the rare times that the sequencer was 'spare' it was used for a special project – the sequencing of the first female genome. Clinical geneticist in training, Marjolein Kriek, who was doing her PhD thesis at the Human Genetics laboratory was chosen as the first woman whose complete genome would be sequenced. 'One of the reasons for choosing her was from the "informed consent" perspective; being a geneticist herself she was perfectly able to comprehend what she consented to. Another reason was a bit of a pun; the group was thinking of a candidate (and even the 'Millionaires Fair' was mentioned for finding a wealthy sponsor or candidate) until one of our researchers, a New Zealander called Stephan White, commented "After Watson – time for Kriek". That settled it. Today, when you Google the name 'Marjolein Kriek', you get 130,000 hits. She is often asked to present to societal discussions about what genomics has to offer – and what it does not.'

BEN OOSTRA, ERASMUS MEDICAL CENTER ROTTERDAM

CV

Ben Oostra is professor of Molecular and Clinical Genetics at the Erasmus MC of the Erasmus University. He received his MSc in 1977 and his PhD in 1981 in Biochemistry from Groningen University, The Netherlands on Regulation of ribosomal RNA synthesis in Escherichia coli. He completed postdoctoral fellowships on oncogenesis of Polyoma and Adeno virus genes at National Institute of the Medical Research, Mill Hill, London UK and at the Laboratory for Medical Biochemistry, Leiden University prior to accepting the position at the Erasmus University in 1985. His research focuses on genetic molecular and biochemical studies in neurogenetic disorders with specific interest in mental retardation and Parkinson disease. A second interest is on genetics of complex disorders, specifically in isolated populations.

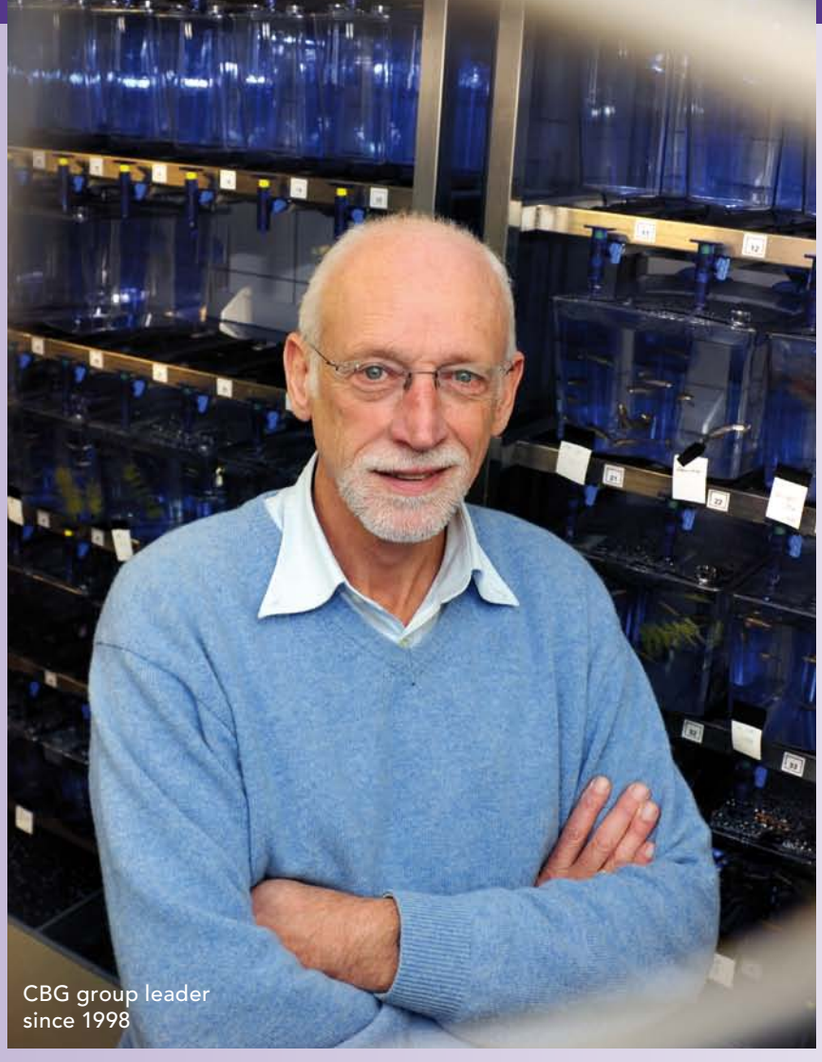
Towards a cure for monogenetic me

Many genes are involved in the development and functioning of the human brain. Due to the complexity and inaccessibility of the brain, the relationship between genetic defects, specific cerebral pathology and neurological or psychiatric symptoms can be hard to establish. Ben Oostra and his group are at the forefront of neurogenetics, studying both monogenetic syndromes like fragile X mental retardation and the more complex development of diseases like multiple sclerosis and Alzheimer's dementia. 'We employ all kinds of techniques, from epidemiology to transgenic and knockout animal models. But the classical, medical genetic approach is always the foundation of our work.'

'Collaboration is very important to us', Oostra says. 'We work together with the neurobiology group of Chris de Zeeuw here in Rotterdam, and with my colleague, geneticist GertJan van Ommen in Leiden, with whom we share the genetic approach. So I'm very happy to participate in CBG and the Centre for Medical Systems Biology (CMSB). Some of the research we do comes with a high cost, like some of the animal models we've developed and the genome-wide assays. It has been more than worth those costs in terms of scientific gains, but it is only possible with sufficient funding.'

Complex disorders

To find the genes involved in complex diseases, an epidemiological approach is needed. In population studies, however, the correlations are often relatively weak. That is why Oostra and his colleague Van Duijn of the Genetic Epidemiology group prefer to study genetic factors in the relatively closed community of Rucphen, which



CBG group leader since 1998

Goals

Studying the genetic basis of monogenic human diseases with specific focus on a number of 'brain' disorders, and the (dys)function of the gene(s) involved. Studying genetic factors involved in the etiology of various complex genetic disorders.

Facilities and expertise

Proteomics: equipped with several mass spectrometers and an expert group running the facility. Genomics: equipped with a diverse set of machinery of which the array and massive parallel sequencing technologies are the most important. The output has been coupled to an efficient bio-informatics platform. Imaging: a number of microscopes are operational within this facility, ranging from confocal microscopes to TIRF and 4π microscopy. Zebrafish facility to study function of disease genes.

ntal retardation

Oostra describes as an 'isolate': 'It's a community of 20,000 people founded by about a hundred, without much intermingling with anyone from the outside. The genetics of such a community are less complex than in the general population because the variation is smaller. If there are twenty genetic risk factors of a disease in general, there may be only ten in our isolate.' The Rotterdam geneticists also charted the family relationships of 3000 inhabitants, tracing their family connections back to the 18th century. Together with a thorough medical check-up and of course a genetic profile (covering 350,000 single-nucleotide polymorphisms or SNPs), a valuable dataset was established. This dataset can be used to study various diseases, ranging from multiple sclerosis to metabolic syndrome, Alzheimer's and Parkinson's disease.

'We have contributed to the practical application of the gene we discovered. That was really gratifying. But we also have an obligation to basic science, to go on finding new mechanisms.'

An important finding in the Rucphen community has been the association between multiple sclerosis and the *KIF1B* gene in the central nervous system. It may well explain the susceptibility to lasting neurological damage (axonal loss) in multiple sclerosis – until then, the disease had only been associated with genes involved in the immune response, confirming the role of the immune system in the disease. Starting with an analysis of about 45 multiple sclerosis patients and 195 controls from the Rucphen isolate, the researchers proceeded to search for novel genes in a larger sample of patients with the disease (sporadic cases

BEN OOSTRA, ERASMUS MEDICAL CENTER ROTTERDAM

Key publications

1 Njajou OT, Vaessen N, et al (2001). A mutation in SLC11A3 is associated with autosomal dominant hemochromatosis. Nature Genet 28:213-214.

2 Zalfa, F., Giorgi, M., et al (2003). The Fragile X Syndrome Protein FMRP Associates with BC1 RNA and Regulates the Translation of Specific mRNAs at Synapses, Cell 112, 317-327.

3 Willemsen, R., Hoogeveen-Westerveld, et al (2003). The FMR1 CGG repeat mouse displays ubiquitin-positive intranuclear neuronal inclusions; implications for the cerebellar tremor/ataxia syndrome., Hum Mol Genet 12, 949-959.

4 Bonifati V, Rizzu P, et al (2003). Mutations in the DJ-1 gene associated with autosomal recessive early onset Parkinson. Science 299, 256-259.

5 Pietrobono R, Tabolacci E, et al (2005). Molecular dissection of the events leading to inactivation of the FMR1 gene. Hum Mol Genet 14:267-277.

6 Koekkoek SK, Yamaguchi K, et al (2005). Deletion of FMR1 in Purkinje Cells Enhances Parallel Fiber LTD, Enlarges Spines, and Attenuates Cerebellar Eyelid Conditioning in Fragile X Syndrome. Neuron 47:339-352.

7 Spencer CM, Serysheva E, et al (2006) Exaggerated behavioral phenotypes in Fmr1/Fxr2 double knockout mice reveal a functional genetic interaction between Fragile X-related proteins. Hum Mol Genet 15:1884-1894.

8 Aulchenko YS, Hoppenbrouwers IA, et al (2008) Genetic variation in the KIF1B locus influences susceptibility to multiple sclerosis. Nat Genet 40:1402-1403.

9 Zhang J, Fang Z, et al (2008). Fragile X-Related Proteins Regulate Mammalian Circadian Behavioral Rhythms. Am J Hum Genet 83:43-52.

10 Aulchenko YS, Ripatti S, et al (2009). Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts. Nat Genet 41:47-55.

and patients from families with two or more patients). The findings were confirmed in a larger Swedish cohort. Oostra summarises: 'With our data on kinship, relatively small effects still can be detected. You see, to study a *single* gene in depth is quite an enterprise. So the more information you can gather from the epidemiological approach, the better you can focus on the genes that matter.'

Fragile X syndrome

The work of Oostra and his co-workers on fragile X syndrome shows very clearly that the study of a single gene can be exciting enough. After their discovery of the fragile X mental retardation-1 gene *(FMR1)*, they proceeded to study the effects of its protein, FMRP, using tissue culture and transgenic mice and material from human patients. They even have come close to the ultimate dream of anyone studying a severe hereditary disorder – a cure! Or at least a way to ameliorate the symptoms caused by the absence of FMRP in fragile X syndrome. Oostra explains: 'FMRP acts as a regulator of mRNA transport. It also regulates the translation of target mRNAs at the synapse upon stimulation of the metabotropic glutamate (mGlu) receptor. The absence of FMRP causes upregulation of translation of several mRNAs, interfering with the function, plasticity and microscopic morphology of the synapse. One of the major consequences is that if you reduce the mGlu signal with an antagonist, you get a partial restoration of function.' The Rotterdam researchers demonstrated this not only in cell cultures, but also on a behavioural level in transgenic mice. Within half an hour of administration, their performance on the test was within the same range as healthy controls. Clinical tests in fragile X patients using similar behavioural tests are underway. Oostra hopes that medication will reduce the number of seizures in patients and that it will have a positive effect on the anxiety and hyperactivity they experience – maybe even on their intelligence. 'I do not expect it to bring their IQ within the normal range, but early administration may make the difference between an institutionalised life and relative independence. If treatment works, early detection of fragile X in neonates may become opportune, so children could have maximum benefit from medication.'

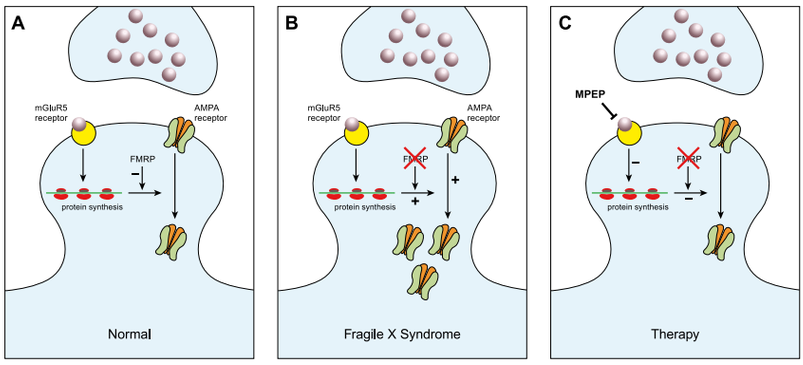
Current group members

Staff
Vincenzo Bonifati
Andre Hoogeveen
Annelies de Klein
Mark Nellist
Arnold Reuser
Rob Willemsen

Postdocs
Edward Mientjes
Femke de Vrij
Aida Bertoli-Avallo
Sima Kheradmand Kia
Renate Hukema

PhD students
Vanessa French
Celine de Esch
Martje Visser
Andrea Pop
Marialouisa Quadri
KateYonova
Danielle Veenma
Tianna Zhao
Liesbeth de Jong
Francesca Punzo
Josien Levinga
Hanneke Mensink
Ingrid van der Laar
Yvonne Hoedemaekers
Najaf Amin
Maaike Schuur
Maxim Struchalin
Ause Demirkan
Linda Broer

Technicians
Bianca de Graaf
Christan Rohe
Guido Breedveld
Erik Simons
Leontien van Unen
Ingeborg Nieuwenhuizen-Bakker
Lies-Anne Severijnen
Herma Zondervan
Ronald Buijsen
Bert Eussen
Hannie Douben
Marianne Hoogeveen-Westerveld
Marian Kroos



The mGluR theory. A hypothetical model for the action of fragile X mental retardation protein (FMRP) at the synapse.

Treatment with MPEP an mGluR5 antagonist, results in the rescue of some phenotypic features because mGluR5 stimulation is reduced and subsequently local translation at the synapse is no longer exaggerated. Ultimately, the number of internalized AMPAreceptors is reduced and restored to normal levels. (A) Stimulation of mGluR5, a metabotropic glutamate receptor, induces local mRNA translation. This results in novel protein synthesis that in its turn stimulates the internalisation of the ionotropic AMPA receptor, essential for long-term plasticity. FMRP acts as a negative regulator of transcription, reducing the internalisation of the ionotropic glutamate receptor. (B) In neurons from fragile X patients the absence of FMRP leads to increased internalisation of ionotropic glutamate receptors, which results in enhanced long-term depression (LTD). (C) Rescue of normal translation due to the mGluR antagonist MPEP, slowing down the internalisation of the ionotropic glutamate receptors.

Fragile X-associated tremor/ataxia syndrome

Oostra's group has begun to tackle yet another disease caused by the *FMR* gene: fragile X-associated tremor/ataxia syndrome or FXTAS, which develops later in life. Oostra says: 'We are using mouse models and fibroblast-derived stem cells from human patients to understand this disorder better.

Using fibroblast-derived stem cells may also help to solve another riddle: how the expansion of the CGG repeat in fragile X develops. Normally, there are 5–55 CGG repeats in the *FMR1* gene. Then there is the pre-mutation of 55–200 repeats that causes FXTAS. Nobody knows what causes this expansion that leads up to the full mutation of more than 200 repeats. 'Stem cells may help us to study all the steps of the expansion. In the past few years, we have contributed to the practical application of the gene we discovered. That was really gratifying. But we also have an obligation to basic science, to go on finding new mechanisms. One of those may be the first lead to another treatment in the future.'

TITIA SIXMA, THE NETHERLANDS CANCER INSTITUTE AMSTERDAM

CBG group leader
since 2004

Enzymes at work – see them with your own eyes

People like to see things with their own eyes. Although science has many instruments for measuring and calculating what happens in small vessels and tubes, and scientists know how easily the eye can be deceived, they still like to 'see' the object of their scrutiny. Imaging of molecules in atomic detail is a major feature of Titia Sixma's work. Her research at the Division of Biochemistry at the Netherlands Cancer Institute (NKI) involves crystallising and analysing proteins involved in the development and maintenance of cancer – in order to understand their function. Her work on the nicotinic receptor made her famous.

Crystals are crucial for detecting the three-dimensional structure of proteins with crystallography. The technique relies on analysis of the scattering of a high-powered beam of x-rays by the electrons in a crystallised molecule. The pattern formed reveals the location and surroundings of individual atoms in the protein. Knowing the primary structure of the investigated molecule and indepth analysis of the scattered beam inform the crystallographer about how the molecule has been folded – that is, its spatial structure. The better the proteins are ordered in the crystal, the more detailed are the results. And those results are now used to create amazingly colourful and intriguing pictures on a screen.

A layman may recognise the hand of an abstract artist, but only expert crystallographers like Sixma

CV

Titia Sixma received her PhD training in the group of Wim Hol at Groningen University studying structure and function of a enterobacterial homolog of cholera toxin, LT. She worked as a post-doctoral fellow in the group of Paul Sigler at Yale University. Since 1994 she has worked as group leader in the Netherlands Cancer Institute in Amsterdam, using structural biology as a tool to study the regulation of signaling processes within the cell. The group has mainly focused on the process of DNA mismatch repair, the analysis of the proteins involved ubiquitin conjugation and studies of a homolog of the nicotinic acetylcholine receptor. Since 2004 she has a part-time appointment as professor of protein structure and function at the Erasmus University in Rotterdam.

Goals

To understand fundamental processes at the atomic level by combining X-ray crystallography provides with a variety of biochemical and biophysical techniques. The focus is on processes in ubiquitin conjugation and DNA repair, as well as ligand-binding in cys-loop receptors. These studies provide mechanistic insight in the molecular processes and provide targets for drug design studies.

Facilities and expertise

High throughput protein expression and purification, automated nanocrystallization, structure analysis by X-ray crystallography, biophysical characterization of protein interactions using isothermal calorimetry, surface plasmon resonance, multi-angle laser light scattering, thermal melting, analytical gelfiltration and fluorescence anisotropy.

can see, by zooming and rotating these pictures, interesting features and connections in the countless forks and ribbon-like structures. 'There seems to be a huge gap between structural chemistry and biology', Sixma says, 'but the interesting connection is – provided that you succeed in unravelling the structure of a protein or the interaction of a protein with another molecule, like a part of the DNA – that you can actually see how the biology works. We see how a protein will cut and repair a mismatch in the DNA helix.'

Nicotine receptors and cancer

Linking the nicotine receptor to cancer via nicotine (the basis of addiction to smoking tobacco and lung cancer) is a bit of a long-shot, admits Sixma. 'The use of drugs that bind to the nicotine receptor may help people who participate in smoking-cessation programmes and thus help prevent cancer. But on top of that, the nicotine receptor family is one of the major targets for drug development in the pharmaceutics industry. Knowing the structure of this receptor is a tremendous step forward for modelling molecules that can bind to it or interfere with the binding of other substances.'

'We can actually see how the biology works. We see how a protein will cut and repair a mismatch in the DNA helix.'

Ubiquitin and DNA repair

Apart from the nicotine receptor, Sixma and her group members have also focused on DNA repair and the conjugation of ubiquitin. The repair of mismatches of DNA bases is crucial to preventing cancer. Families who have mutations in genes involved in DNA repair are highly susceptible to cancer, especially colon cancer. Sixma's group investigates the coupling of the repair-protein complex and the DNA mismatch, and the involvement of energy-supplying molecules like adenosine triphosphate (ATP). They can now show the whole sequence of the process on their computer screens and assess each step in the process. 'It is fascinating to see how smartly the molecular

TITIA SIXMA, THE NETHERLANDS CANCER INSTITUTE AMSTERDAM

Key publications

1 Lamers, M.H., Perrakis, A., et al (2000). The crystal structure of DNA mismatch repair protein MutS binding to a G:T mismatch. Nature 407, 711-717.

2 Brejc, K., van Dijk, W.J., et al (2001). The crystal structure of AChBP, homolog of the N-terminal domain of the nicotinic acetylcholine receptor. Nature, 411, 269-276.

3 Lamers, M.H., Winterwerp H.H.K., Sixma, T.K. (2003). The alternating ATPase domains of the DNA mismatch repair enzyme MutS control DNA mismatch repair. EMBO J. 22, 746-756.

4 Celie, P.H.N, van Rossum-Fikkert, et al (2004). Nicotine and carbamylcholine binding to nicotinic receptors studied by AChBP structures. Neuron, 41, 907-914.

5 Pichler, A., Knipscheer, P., et al (2005). SUMO inhibits the ubiquitin E2 enzyme E2-25K via modification of a helical acceptor site. Nat. Struct. Mol. Biol. 12, 264-269.

6 Nijman S.M., Luna-Vargas M.P.A., et al (2005). Genomic and Functional Inventory of Deubiquitinating Enzymes, Cell 123, 773-786.

7 Ulens, C., Hogg, R.C., et al (2006). Structural determinants of selective a-conotoxin binding to a nicotinic acetylcholine receptor homolog AChBP. Proc. Natl. Acad. Sci. USA, 103, 3615-3620.

8 Buchwald, G., van der Stoop, P., et al (2006). Structure and E3-ligase activity of the Ring-Ring complex of polycomb proteins Bmi1 and Ring1b. EMBO J. 25, 2465-2474.

9 Knipscheer, P., van Dijk, W.J., et al (2007). Noncovalent interaction between Ubc9 and SUMO promotes SUMO chain formation. EMBO J 26(11):2797-2807.

10 Knipscheer, PK, Flotho A., et al (2008). Ubc9 sumoylation regulates SUMO target discrimination. Mol. Cell., 31:371-382.

complex executes its task. The goal of this line of research is mostly mechanistic; we want to understand the process. We do not want to intervene with a drug, as such, because quenching the DNA repair just enhances the cancer risk.'

The ubiquitin investigation, however, definitely has potential for future drug design. When ubiquitin attaches to a protein in cells, it signals that the protein should be transported to a specific site in the cell or should be broken down. Ubiquitin is a small protein of just 76 amino acids, but conjugation of ubiquitins can be very complex. They can form conjugates with proteins but also with other ubiquitin molecules and lots of different structures can be formed, many of which seem to create very different signals – certain chains of four ubiquitin molecules, for instance, can lead to the destruction of the protein concerned.

Sixma's group studies the way in which ubiquitin attaches to and detaches from proteins. Sixma explains: 'This attaching and detaching of ubiquitin, and thus the breakdown of proteins, is a very important step in the regulation of cellular processes. For instance, the activity of a tumour suppressor gene like p53 is highly dependent on the amount of p53 protein available. That availability is regulated by the balance of ubiquination and de-ubiquination of the p53 protein. It is interesting that there are several tumour genes and tumour suppressor genes involved in the process in which ubiquitin is conjugated with proteins. A mechanism that is comparable to that of ubiquitin has been found with SUMO. This protein is involved in the regulation of genes and the regulation of the enzymatic activity of enzymes. These are terribly complicated processes, which makes it fun to study them.'

Successful crystallisation of proteins

The research group is highly instrument-oriented. The key to successful crystallography is the ability of the protein to be crystallised – a property that is not obvious for proteins – so crystallisation is often a process of educated trial and error. Microvessels with solutions containing proteins are brought into equilibrium with different kinds of precipitants in the hope that tiny crystals will be formed. Biochemists can help this process by

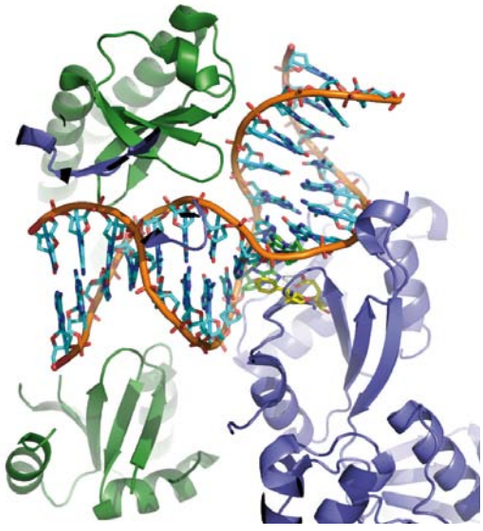
Current group members

Postdocs
Alex Fish
Rick Hibbert
Prakash Rucktooa
Mariano Stornaiuolo
Azusa Seto

PhD students
Mark Luna-Vargas
Alex Faesen
Franceska Mattiroli
Judith Smit
Danny Sahtoe
Flora Groothuizen

Technicians
Herrie Winterwerp
Pim van Dijk

'The nicotine receptor family is one of the major targets for drug development in the pharmaceutics industry. Knowing the structure of this receptor is a tremendous step forward for modelling molecules that can bind to it or interfere with the binding of other substances.'



The crystal structure of DNA repair protein MutS shows how the protein recognizes the mismatches by inserting a phenylalanine (yellow) into the DNA.

varying the experimental conditions, and also by changing the proteins themselves, as they are produced in Escherichia coli or insect cells. They introduce changes into the genetic code in places that they know won't influence the tertiary structure of the protein, but that will facilitate the process of crystallisation. If a crystal is formed and tested, then a larger one is grown and taken to the European Synchrotron Radiation Facility in Grenoble to be exposed to strong and tuneable x-rays.

'You have to be patient to crystallise a protein and analyse the x-ray patterns', Sixma says. 'But once you have the structure of your protein it is sometimes possible to see immediately how it works. It is for example amazing to see how the DNA-mismatch-repair protein pushes the amino acid phenylalanine into the place where the DNA is kinked due to a wrongly inserted base, and how it forms a hydrogen bridge to the mismatched base. And in the nicotine receptor we can point to where and how the tiny nicotine molecule is bound in the huge receptor protein', Sixma says about her work with CBG groups in Rotterdam and Utrecht. 'But more often we need to do functional experiments to really understand the implications of what we see. Our next step will be to find out how the system of ubiquitin and SUMO works in laboratory experiments and to find good markers to monitor the method of conjugation in living cells.'

PETER VERRIJZER, ERASMUS MEDICAL CENTER ROTTERDAM

CV

Peter Verrijzer studied biochemistry at Utrecht University and obtained his PhD from the same university (1992). During his postdoctoral period in the group of Robert Tjian at the University of California at Berkeley, he started his work on the basic mechanisms of gene regulation in animal cells. In 1996 he joined the Imperial Cancer Research Fund in London as a group leader to study the role of chromatin and epigenetic processes in the regulation of development and cell proliferation. In 1999 he became a full professor of Molecular and Cell Biology at the Leiden University Medical Center. In 2004 he moved to the Erasmus Medical Center in Rotterdam, where he is professor and head of the department of Biochemistry. His lab made important contributions to our understanding of the enzymatic machinery that controls gene transcription in development and disease. In their study of developmental gene regulation, his lab integrates biochemistry, proteomics, genomics and genetics. Verrijzer is a member of EMBO.

Accessing the libraries of life

Two metres of DNA are folded into the nucleus of each of our cells, which is a space with a diameter of roughly 10 micrometres. Despite this densely packed DNA, each cell must be able to access the blueprints within the genes that are relevant to its functioning. Other genes that are useless, or which may even be dangerous to the cell, must be carefully switched off. Peter Verrijzer and his biochemistry department study this very finely tuned molecular library, in the hope of gaining new insights into the biology of cancer and the development of stem cells.

'In our research we try to address some of the basic questions of developmental biology: How does a cell switch its genes on and off? By what mechanisms does a cell become a brain cell, a liver cell or a muscle cell? In other words, how can a complete organism emerge from a fertilised egg cell? What we know already are the different levels at which the transcription of DNA is regulated. The main focus of our research has always been the folding of DNA into chromatin and the roles of the proteins involved. What has changed in the last few years is that we have fully integrated developmental biology and '-omics' approaches into our studies. We try to work on as many levels as possible, from basic structural chemistry at the molecular level and (in collaboration with the Sixma lab even the atomic level, up to the level of the whole organism. A growing part of our work is based on proteomics and genomics technology. That is why I am very glad about the investments made possible by the CBG

CBG group leader since 1999

Goal

To understand how expression of the eukaryotic genome is regulated.

Facilities and expertise

State of the art mass spectrometry equipment that has been placed in the Erasmus MC proteomics center. Expertise ranges from biochemistry, developmental genetics of *Drosophila*, and genomics approaches to studying gene expression (ChIP-chip, ChIP-seq, genome-wide expression analysis). Also expertise on virtually all techniques required to study transcription control and chromatin dynamics.

and the exchange of people and ideas that it stimulates. And of course, CBG funding facilitates truly innovative research. Other organisations only give funding if you are already almost certain you'll find something; they may claim to stimulate innovation, but they never provide any money for trying something really new.'

'The organism we do most of our research on is the fruit fly – which makes it relatively easy to keep an overview. In the fruit fly we can combine biochemistry, proteomics, genomics and developmental genetics far more easily than if we used other models, like mice. And of course, we can maintain many thousands of fruit flies for the price of a single mouse. The basic mechanisms we study have been conserved throughout evolution (some of them can even be found in yeast cells) and everything we study is also present in mammalian cells. So by conducting research in fruit flies we hope to find mechanisms that are relevant to human medicine, especially cancer and stem cells. The more we find out about chromatin, the easier it will be to deal with cell development and cell growth.'

'How does a cell switch its genes on and off? By what mechanisms does a cell become a brain cell, a liver cell or a muscle cell?'

Malignant tumours

One of the lines of enquiry in Verrijzer's group has always been the role of energy-dependent chromatin remodelling complexes in the regulation of DNA transcription during development and disease.

'More specifically, we study the SWI/SNF-related complexes, a group of molecular motors engaged in moving the nucleosomes along the DNA. You often read that SWI/SWF is involved in opening up chromatin, but we demonstrated that it can also work the other way round. Human SNF-5 is a universal SWI/SNF subunit. It is also a tumour suppressor. If it does not function, a rare but highly aggressive type of cancer will result, called malignant

PETER VERRIJZER, ERASMUS MEDICAL CENTER ROTTERDAM

Key publications

1 Kal AJ, Mahmoudi T, Zak NB and Verrijzer CP (2000). The Drosophila Brahma complex is an essential coactivator for the trithorax group protein Zeste. Genes Dev 14: 1058-1071.

2 Katsani KR, Arredondo JJ, et al (2001). A homeotic mutation in the trithorax SET domain impedes histone binding. Genes Dev 15: 2197-2202.

3 Mahmoudi M, Katsani KR and Verrijzer CP (2002). GAGA can mediate enhancer function in trans by linking two separate DNA molecules. EMBO J 21: 1775-1781.

4 Oruetxebarria I, Venturini F, et al (2004). p16INK4a is required for hSNF5 chromatin-remodeler induced cellular senescence in malignant rhabdoid tumor cells. J Biol Chem 279: 3807-3816.

5 Van der Knaap JA, Kumar BRP, et al (2005). GMP Synthetase Stimulates Histone H2B Deubiquitylation by the Epigenetic Silencer USP7. Mol Cell 17: 695-707.

6 Vries, RGJ, Bezrookove V, et al (2005). Cancer–associated mutations in chromatin-remodeler hSNF5 promote chromosomal instability by compromising the mitotic checkpoint. Genes Dev 19: 665-670.

7 Mohd-Sarip A, Cléard F, at al. (2005). Synergistic Recognition of an Epigenetic DNA Element by Pleiohomeotic and a Polycomb Core Complex. Genes Dev 19: 1755-1760.

8 Mohd-Sarip A, van der Knaap JA, et al (2006). Architecture of a Polycomb Nucleoprotein Complex. Mol Cell 24: 91-100.

9 Lagarou A, Mohd-Sarip A, et al (2008). dKDM2 couples histone H2A ubiquitylation to histone H3 demethylation during Polycomb group silencing. Genes Dev 22: 2799-2810.

10 Moshkin YM, Kan TW, et al (2009). Histone Chaperones ASF1 and NAP1 Differentially Modulate Removal of Active Histone Marks by LID-RPD3 Complexes during NOTCH Silencing. Mol Cell 35: 782-793.

rhabdoid tumour (MRT). Affected children are often born with large tumours and they die before the age of 3 years. We study MRT because we hope that the pathways involved will help us understand other more common forms of cancer.'

Interestingly, his research group also established a close link between SWI/SNF and a known inhibitor of the cell cycle – the INK4b-ARF-INK4 – which is a tumour suppressor locus. Verrijzer explains: 'In MRT cells, SWI/SNF *should* mediate the eviction of Polycomb group silencers and epigenetic reprogramming of this locus, thus activating the p16INK4a tumour suppressor pathway. But in MRT cells SWI/SNF does not function properly and as a result, the p16INK4a anti-cancer function remains dormant.'

The Polycomb group (PcG) proteins are another focus of research for the Rotterdam group. First discovered in fruit flies, they have been demonstrated to be important in many aspects of mammalian cell development. Without crucial PcG genes, the organism cannot survive. Overexpression of PcG genes correlates with the severity and invasiveness of several types of cancer. Verrijzer continues: 'PcG proteins are able to silence genes by changing chromatin structure. We are trying to find out how these proteins find their target genes and how they manage to silence them. We have discovered several interesting aspects already. One of them is the architecture of a nucleoprotein complex we have called a silenceosome, which is in many aspects surprisingly similar to the enhanceosomes that accelerate a gene's transcription – the opposite of silencing. We have determined the different proteins involved in this silenceosome complex and the DNA pattern it binds to. We have also found that there is a much greater diversity among PcG complexes than previously anticipated. So I suppose Polycomb group genes will keep us busy for a while longer.'

Ubiquitin

A relatively recent area of interest for Verrijzer and his colleagues is mono-ubiquitination of histones, the essential protein ingredient in chromatin. 'In most proteins, ubiquitination means that they are marked for destruction

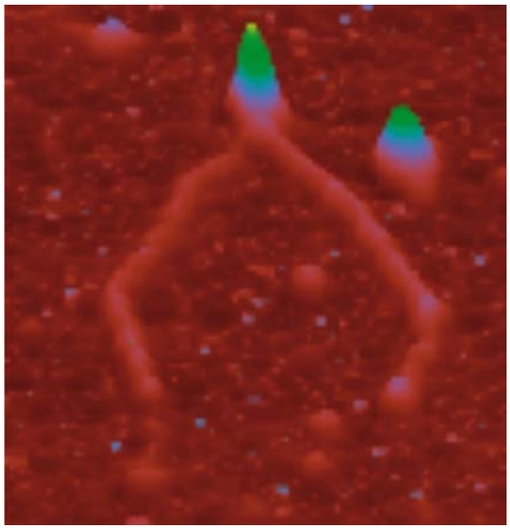
Group members

Postdocs
Jan van der Knaap
Adone Mohd-Sarip
Yuri Moshkin
Stavros Giannakopoulos
Cecile Doyen

PhD students
Ashok Reddy
Elena Kozhevnikova
Olaf Voets

Technicians
Gillian Chalkley
Karin Langenberg
Tsung Wai Kan
Ulku Aslan

in the proteasome. But in histones, the role of ubiquitination is quite different – it is used to switch genes on or off. Interestingly, histones were the first proteins in which ubiquitination was demonstrated, way back in 1975, but there still are many questions about its physiological consequences. Studying one of the enzymes that removes ubiquitin from histones, we made a remarkable discovery. We purified it as a heteromeric complex with guanosine 5'-monophosphate synthetase (GMPS) and found that it contributes to epigenetic gene silencing by histone H2B de-ubiquitylation. Association with GMPS was essential for H2B de-ubiquitylation. So here we have a common biosynthetic enzyme fulfilling all the criteria of a transcription factor: it binds to chromatin on specific genes and it controls their transcription. Now we believe this might well be a trick that nature employs quite often. It would be an interesting link between the metabolic state of the cell and gene expression. We are following up on this line of enquiry and our preliminary results suggest that we may well be on to something promising.'



Scanning force microscopic image of a Polycomb complex bound to its target DNA sequence.

PETER VAN DER VLIET, UNIVERSITY MEDICAL CENTER UTRECHT

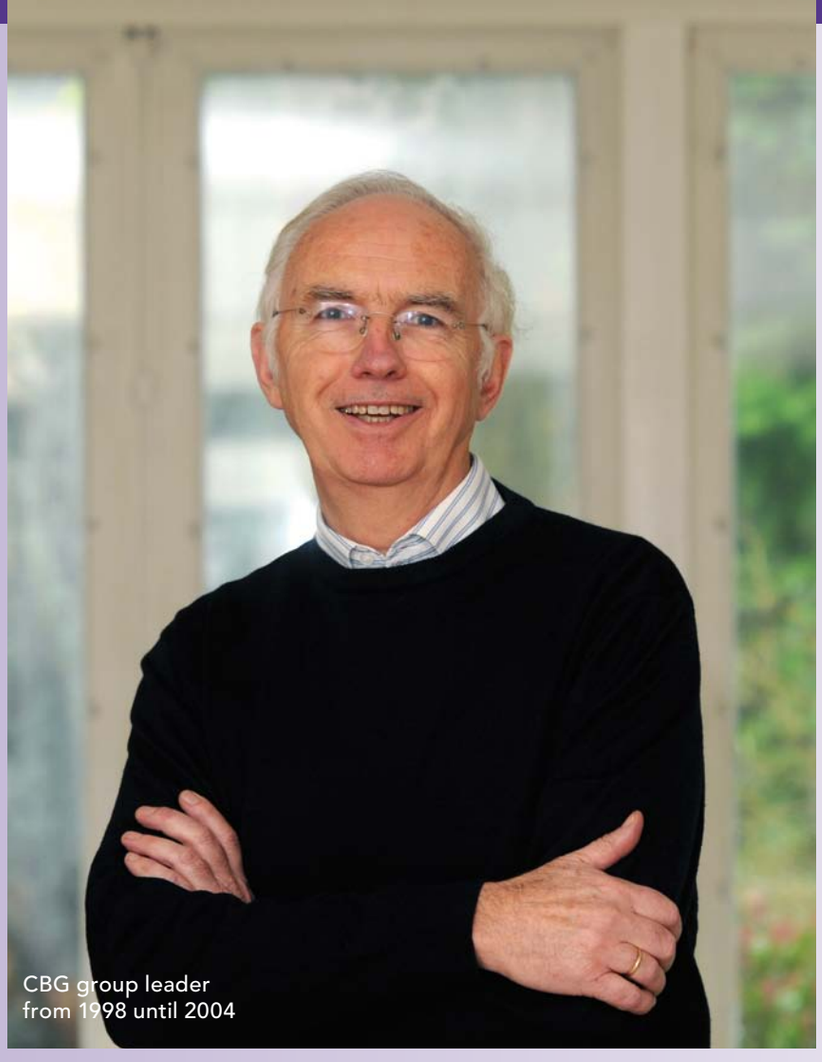
CV

Peter van der Vliet studied biochemistry at Utrecht University (thesis 1971). After a postdoc at Princeton University with Arnold Levine he became full professor in Utrecht in 1984. His main interest are DNA replication and gene expression, using viral models like Adenovirus. He was Chairman of the Department and the Division Biomedical Genetics at the University Medical Center Utrecht and Director-General of the CBG until his retirement in 2006. He has been involved in many scientific boards and committees, both national and international such as the EMBO council and the Royal Netherlands Academy of Arts and Sciences (KNAW) of which he has been been Vice-President. Presently he is chairing, among others, an ALLEA (All European Academies) working group on Evaluation of Science.

A very fruitful approach

In 1998 Peter van der Vliet was one of the founding fathers of the CBG. As Director General of the CBG (until 2006) his primary goal was to make the CBG a success by investing in the most recent technology, by intense collaboration between the participants and the organisation of high-level meetings. Looking back now he concludes that 'our approach turned out to be very fruitful, in terms of scientific output as well as in terms of organisation.' An important ingredient of this success is 'the high degree of mutual confidence within the CBG.'

Being one of the founding fathers of the CBG, Peter van der Vliet feels obliged not only to review some of the scientific highlights of his own group in the first decade of the CBG, but also to look back at the way the CBG is organised and what came out of that. 'The founding of the CBG was the result of the so-called 'In-Depth Strategy' of the Dutch minister of education, culture and science in 1997. He recapitulates: 'It aimed to provide a limited number of research schools, or national research combinations, consisting of groups selected from such schools, with additional funding. This was meant to create so-called 'top schools' that could become or strengthen their status as an international centre of excellence. As a group of researchers from the universities of Utrecht and Rotterdam as well the Netherlands Cancer Institute in Amsterdam, all working within the field of life sciences, we decided to collaborate and make a joint proposal. The research collaboration we had in mind would



CBG group leader
from 1998 until 2004

Goals

Understanding the way in which DNA duplicates, employing adenovirus as a model; The use of advanced structural analysis to study protein-DNA interactions involved in DNA replication; As Director-General, making the CBG a success by investing in the most recent technology, intense collaboration between the participants and the organisation of high-level meetings

Facilitiesand expertise

Protein purification and structural analysis; *In vitro* DNA replication systems; DNA replication enzymes; Protein-DNA interactions; Transcription factors.

explore in full detail the function of genes and gene products in relation to disease. Therefore, the members of this collaboration had to combine a wide range of expertise required to carry out the research program, spanning genetics, molecular biology, cell biology, as well as biochemistry and structural biology. We decided to do the toughest job first – to select who was in and who wasn't. The potential members of the organisation, which we named the Centre for Biomedical Genetics, were first and foremost chosen based on their scientific track record, and their area of research had to fit in with the mission of the CBG. Somewhat later, Leiden University also joined and we finally able to select 15 members. There were five from Utrecht and Amsterdam, three from Rotterdam and two from Leiden. We also decided to make firm agreements in advance about the way the CBG's funds would be spread among members. Looking back now, I can say that this approach has turned out to be very fruitful. By choosing all members primarily on proven scientific quality, we created great mutual confidence and complementarity within the CBG. Because of this mutual confidence we could offer members maximum freedom for spending the funds. Unlike other grants, money provided by the CBG only had to be accounted for *afterwards* – not in advance. This provided enough flexibility for members to react quickly on new developments in their field of research, perhaps by investing in a new technique or by appointing an extra investigator to boost a certain line of research.'

'We were able to identify and characterise all the proteins involved in the replication of viral DNA.'

'Moreover, the firm agreements we made in advance made it possible to 'run' the CBG with very limited overheads. As a result, the funds could almost entirely be invested in research and research-related subjects, for example organising meetings.'

'I think it's fair to say that the CBG proved it is possible to organise scientific research in a very

PETER VAN DER VLIET, UNIVERSITY MEDICAL CENTER UTRECHT

Key publications

1 Bas van Breukelen, Paul A. Tucker et al (2000). The formation of a flexible DBP protein chain is required for efficient DNA unwinding and Adenovirus DNA chain elongation. J. Biol. Chem., 275, 40897-40903.

2 Brenkman AB, Heideman MR et al (2001). The (I/Y)XGG motif of adenovirus DNA polymerase affects template DNA binding and the transition from initiation to elongation.J. Biol.Chem. 276, 29846-53.

3 R.N. de Jong, M.E. Mysiak et al (2002). Recruitment of the priming protein pTP and DNA binding occur by overlapping Oct-1 POU homeodomain surfaces. EMBO J. 21, 725-735.

4 Arjan B. Brenkman, Elise B. Breure and Peter C. van der Vliet (2002). Adenovirus DNA polymerase: binding of the protein-primer and location of the active sites. J. Virol. 76, 8200-8207.

5 Kevin D. Augustijn, Dawn L. Duval et al (2002). Structural characterisation of the Pit-1/Ets-1 interaction: charge dependence of Pit-1 for Ets-1 binding. Proc.Natl.Acad. Sci USA 20, 12657-12662.

6 Bas van Breukelen, Arjan B. Brenkman et al (2003). Adenovirus type 5 DNA binding protein stimulates binding of DNA polymerase to the replication origin, J.Virol.77, 915-922.

7 R.N. de Jong, A.B. Brenkman and P.C. van der Vliet (2003). Adenovirus DNA replication: Protein priming, jumping back and the role of the DNA Binding Protein DBP. Curr. Top. Microbiol. Imm. 272, 187-213.

8 M.E. Mysiak, M.H. Bleijenberg et al (2004). Bending of Adenovirus origin DNA by Nuclear Factor I as shown by Scanning Force Microscopy is required for optimal DNA replication. J. Virol, 78, 1928-1935.

9 Mysiak M.E., Wyman C. et al (2004). NFI and Oct-1 bend the Ad5 origin in the same direction leading to optimal DNA replication. Nucleic Acids Res. 32, 6218-6225.

10 Peter C. van der Vliet and Rob C. Hoeben, Adenovirus, in "DNA Replication and Human Disease", Ed. DePamphilis, M.L., Cold Spring Harbor Laboratory Press 2006, 645-661.

low-key manner, provided that there is enough mutual confidence and trust in the scientific integrity of the members.'

Investment and collaboration lead to success

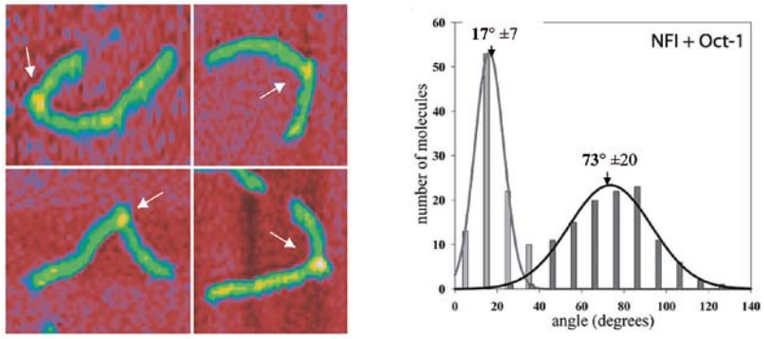
In terms of organisation, as well as output, the CBG can be described as successful. This was the conclusion of Van der Vliet at the 10th anniversary of the CBG. 'We made a lot of investments in technology platforms that are shared by all members, for example mass spectroscopy, microarray facilities and large-scale cell culture, as well as mouse mutant facilities, bioinformatics and the creation of an RNA interference (RNAi) library'. He starts to enumerate: 'There has been an increase in the collaboration between members. Each CBG group collaborates with at least three other CBG groups. The scientific production, in terms of publications of CBG members in high impact papers like *Nature* and *Science*, is about 20 percent higher than it was before the start of the CBG. Disseminating the results of the research has been done efficiently, by means of annual meetings at the Royal Tropical Institute, for example, which are attended by hundreds of scientist from all over the world. The CBG has also catalysed new centres of excellence like the Cancer Genomics Center in addition to several start-up companies. Moreover the CBG provides a lot of young investigators with an opportunity to build up a personal track record. Some of them have become members of the CBG themselves since.'

DNA bending and replication

Apart from being Director General of the CBG, Van der Vliet was also involved in the CBG as a researcher. Until his retirement in 2006 he was head of the Department of Physiological Chemistry and the Division of Biomedical Genetics at the University Medical Center Utrecht. His research focused on the most fundamental process of life – DNA replication. 'Every living cell has to duplicate its DNA before it can multiply. DNA replication is an extremely complex process. It has to been done at a high speed and without mistakes. Throughout my scientific career I have been studying the molecular details of the process, mostly using the adenovirus as a model. With this model it is possible to reconstruct DNA replication completely *in vitro*, and we were able

'Moreover the CBG provides a lot of young investigators with an opportunity to build up a personal track record.'

What happens when NF1 and Oct-1 are simultaneously bound to the origin?



Bending of the origin by the combined action of Oct-1 and NFI as shown by scanning force microscopy. The histogram represents the DNA bend angle distribution of the protein-free DNA molecules (light grey bars) and the DNA-NFI-Oct-1 complexes (dark grey bars). SFM images of the protein-DNA complexes are shown. The location of the origin is indicated with arrows. See Mysiak et al. 2004.

to identify and characterise all the proteins involved in the replication of viral DNA. We showed that the virus uses viral proteins (for example DNA polymerase and DNA-binding protein) as well as proteins belonging to the host cell. The latter unexpectedly turned out to be transcription factors; one of these later proved to be a member of a family of factors now known as the 'octamer' binding family. One particular member of that family, Oct-4, is currently gaining much attention as an important protein in cell de-differentiation. Two of the transcription factors, nuclear factor 1 (NF1) and Oct-1, are involved in adenovirus DNA replication and bind simultaneously to the so-called origin of replication within the adenovirus DNA, thereby enhancing initiation of replication. In collaboration with Rob Kaptein's group, we managed to elucidate the three-dimensional structure of Oct-1 and to show how it recognises DNA. We used scanning force microscopy in collaboration with Claire Wyman (a member of Jan Hoeijmakers' group) in order to propose a model that explains the enhancement of replication initiation after binding of the transcription factors. Binding of Oct-1, as well as binding of NF1 proved to bend the DNA at the binding site. Because NF1 and Oct-1 bind to the DNA strand in close proximity of each other, excessive bending of the DNA takes place at the binding site. It is this conformational change that facilitates binding of the DNA polymerase, and thus brings about DNA replication.'

CBG 1998–2009

Mission

To understand the function of genes and gene products in relation to disease, employing a multidisciplinary approach.

4 Research Themes

1 Animal Models and Cancer
2 Molecular Cancer Cell Biology
3 Genome Maintenance and Expression
4 Genetics of Neurological and Muscular Disorders

6 Institutes

1 Netherlands Cancer Institute (NKI), Amsterdam
2 Hubrecht Institute for Developmental Biology and Stem Cell Research (KNAW), Utrecht
3 Erasmus Medical Center Rotterdam (Erasmus MC), Rotterdam
4 Leiden University Medical Center (LUMC), Leiden
5 University Medical Center Utrecht (UMCU), Utrecht
6 Utrecht University (UU), Utrecht

5 Graduate Schools

1 Oncology Graduate School Amsterdam (OOA), Amsterdam
2 Medical Genetics Centre South-West Netherlands (MGC), Rotterdam and Leiden
3 Research School Cancer Genomics & Developmental Biology (CGDB), Utrecht
4 Graduate School Biomembranes (Biomembranes), Utrecht
5 Bijvoet Graduate School for Biomolecular Chemistry (Bijvoet), Utrecht

23 Research Groups

Group Leader	CBG Participation	CBG Theme	Graduate School
Reuven Agami	2009 - Present	3	OOA
René Bernards	1998 - Present	2	OOA
Anton Berns	1998 - Present	1	OOA
Piet Borst	1998 - 2003	2/3	OOA
Johannes Bos	1998 - Present	2	CGDB
Boudewijn Burgering	2004 - Present	2	CGDB
Hans Clevers	1998 - Present	2	CGDB
Peter ten Dijke	2009 - Present	2	MGC
Lex van der Eb	1998 - 2000	2	MGC
Hans Geuze	1998 - 2003	2	Biomembranes
Frank Grosveld	1998 - Present	3	MGC
Albert Heck	2009 - Present	2	Bijvoet/CGDB
Jan Hoeijmakers	1998 - Present	3	MGC
Robert Kaptein	1998 - 2008	3	Bijvoet
Maarten van Lohuizen	2004 - Present	1	OOA
Wouter Moolenaar	1998 - 2008	2	OOA
Jacques Neefjes	2009 - Present	2	OOA
Gertjan van Ommen	1998 - Present	4	MGC
Ben Oostra	1998 - Present	4	MGC
Ronald Plasterk	1998 - 2007	1	CGDB
Titia Sixma	2004 - Present	2	OOA
Peter Verrijzer	2000 - Present	3	MGC
Peter van der Vliet	1998 - 2003	3	CGDB

Junior Scientific Personnel in CBG Research Groups (2008)

111 fte[1] postdoctoral fellows
86 fte PhD students

Expertise in CBG Research Groups

The CBG combines expertise in Genetics, Genomics, Proteomics, Bioinformatics, Molecular and Cell Biology, Biochemistry and Structural Biology.

Budget Allocation

The CBG was established in 1998. During the period 1999–2008, the Centre received on average 3.1 M€ per year from the Dutch Ministry of Science, Education and Culture. The current level of funding is 3.7 M€ per year for the period 2009–2013. Approximately 30% is spent on investments in new technology and the remainder is spent on positions for postdoctoral fellows and PhD students and for annual high-quality scientific meetings.

Investments in New Technology

During its existence, the CBG has invested heavily in new technologies, such as DNA chip and microarray technology, bioinformatics and automation, animal model systems (mouse; *C. elegans*), mass spectroscopy (ESI-TOF; triple-quadrupole MALDI-TOF), large-scale cell culture, FACS flow cytometry, atomic force microscopy (AFM), fluorescence resonance energy transfer (FRET), cryo-electron microscopy, and siRNA libraries.

[1]fte = full time equivalent

Centre for
Biomedical
Genetics