# The Novo Nordisk Foundation Center for Protein Research Annual Report 2013



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### **Executive summary**

2013 was special for CPR in many ways. First and foremost, CPR is coming of age and has reached the point of midterm evaluation after 5 years of existence. In February 2013, CPR hosted an international panel of peer reviewers, who rigorously scrutinized our scientific output, management, career opportunities, integration in the Faculty of Health and Medical Sciences, and the impact of our research on society. Our overall performance was rated as excellent and the key message, which grabs the essence of one of the unique features of CPR, is best summarized in a quote from the midterm assessment: '*The achievements are even greater when one takes into account that most of the group leaders were recruited to CPR only in 2009 or later*'. We would like to add to this that most CPR group leaders run their research groups for the first time in their careers, which makes their achievements truly remarkable and creates a strong foundation for placing CPR on the scientific map of the world. We are very proud of our young group leaders and their teams!

The second crucial outcome of the midterm review was that the Board of the Novo Nordisk Foundation (NNF), motivated by (quote) 'excellent scientific performance and the great potential to further consolidate a prominent position in the international league of protein science' invited the CPR management to develop a long-term strategy for CPR's future development. We see this as an expression of trust by the NNF in our scientific potential, and we have worked hard to develop a strong vision for our research in the coming decade.



To finalize this strategy, operationally called 'Integrative protein technologies as a means to understand complex biological systems' is the number one task for the coming year.

Speaking of evaluations, 2013 was also marked by the inaugural quinquennial group review, an important means introduced by the CPR Executive Director to assess the scientific output of individual group leaders, direct them in their future research careers, and allocate strategic core funds to the most competitive areas of protein science. The first group leader to be evaluated was Lars Juhl Jensen, who heads the Cellular Network Biology Group at the Disease Systems Biology Program. A three-member international panel of peer reviewers scored his scientific performance as 'outstanding', the highest score on a 5-point scale, and recommended to extend the core funding for his group for the next five years. Based on this assessment, the CPR management recommended promotion of Lars Juhl Jensen to Professor with tenure.

These accomplishments would not be possible with-



out hard work leading to a remarkable scientific productivity and high-impact discoveries. Without exception, all groups at CPR contributed to this endeavor and a considerable part of this annual report is therefore dedicated to highlighting our most exciting results and their biological and medical implications.

Here, we wish to highlight the central aspect of these achievements, which nicely illustrates the spirit of the CPR concept: CPR has been conceived as a highly integrated research center, where groups should not compete with each other for funds, space or influence. Instead, CPR scientists are encouraged to exploit the opportunity of working together and benefit from the access to a unique combination of state-of-theart protein technology platforms. Such setup (and mindset) promotes interdisciplinary projects

and thereby increases the potential to obtain a holistic view on fundamental aspects of protein biology. It has been a gratifying experience to see that this concept has become reality and indeed a major source of our success. To highlight one example out of many: 15 primary research articles were published in 2013 by CPR scientists in Cell journals (Cell, Molecular Cell, Cell Reports) and 6 of these were conducted as close collaborations between groups across the CPR research programs.

The notion that CPR is coming of age is further underlined by the increasing success of our scientists to win some of the most competitive external grants and awards. Among the most remarkable achievements in 2013 was the first ERC grant brought into CPR by Niels Mailand, principal investigator of the Ubiquitin Signaling Group at the Disease Mechanisms Program. Niels Mailand also received the Silver Medal for young Danish researchers awarded by the Royal Danish Academy of Science and Letters. Additionally, Niels Mailand and group leader Chunaram Choudhary from the Proteomics Program became EMBO Young Investigators in 2013. Importantly, the attitude and success of CPR scientists in competing for prestigious grants and awards is not restricted to principal investigators: A Sapere Aude Award to Matthias Altmeyer and the EU Marie Curie fellowships to Andreas Mund and Kai Neelsen are among the notable examples of very successful postdoctoral career development.

Another highlight of 2013 is the CPR expansion. With the growing potential of the CPR technological pipeline and thanks to the fact that CPR is well integrated in the Faculty of Health and Medical Sciences, we have become a sought-after destination for very talented PhD students and postdocs. To become a truly comprehensive protein center and further increase our potential to educate protein researchers, we must strengthen our efforts in understanding protein structure and move from isolated proteins towards complex protein machines - the true effectors of cellular functions. We were very fortunate to attract Guillermo Montoya, an international leader in structural biology, to establish a strong research group and lead the newly established Protein Structure Program at CPR. From January 2014, Guillermo will move to Copenhagen from his current position as senior investigator at the Spanish National Cancer Center (CNIO) in Madrid. We were also successful in strengthening technological expertise in protein imaging by the recruitment of Jutta Bulkescher. Jutta provides the highest level of technical support to the Protein Imaging Facility shared between CPR and DanStem, a neighboring NNF-funded center. Jutta was trained at the European Molecular Biology Laboratory (EMBL) in Heidelberg and is without exaggeration one of the most experienced technicians in the field of advanced microscopy.

The achievements are even greater when one takes into account that most of the group leaders were recruited to CPR only in 2009 or later...

Last but not least, although we highlighted CPR management and principal investigators in the previous paragraphs, we would like to end the introduction to this annual report by emphasizing the CPR employees generating the real value in the form of remarkable scientific discoveries: Our students and postdocs, with help and support from the technical and administrative staff. We appreciate their dedication and enthusiasm and will continue to look for every opportunity to help them realize their scientific dreams, interact with their peers, and find new opportunities for shared projects and collaborations.

The most successful initiative in this regard was the introduction of our 'CPR Research in Progress' (CPR-IP) meetings, focused on sharing new and unpublished results. The CPR-IP meetings are organized monthly in the newly renovated Faculty Club, located on the top of the faculty building with a stunning view over the city of Copenhagen.

This venue has somehow created the 'genius loci' because the talks and subsequent discussions are equally or more interesting than those on established high-profile 'senior' scientific conferences. The CPR-IP meetings have already proven the potential to spark exciting interdisciplinary projects involving groups across all CPR programs.

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Jiri Lukas **Executive Director** 

Jesper Velgaard Olsen Vice Director

Peter Dyrsting Head of Administration

### **Recruitment of Professor Guillermo Montoya**

The structural understanding of proteins involved in disease-related processes is an area of research with increasing importance for CPR. Structural Biology and the associated key protein technologies are now being established at CPR at a very high level thanks to the recruitment of Professor Guillermo Montoya as new research director, starting in 2014. The establishment of a strong program under Professor Montoya's leadership is a natural prolongation of our protein production and characterization effort, which has been a core mandate of CPR since its beginning.

Professor Montoya joins CPR from a position as Senior Group Leader at the Spanish National Cancer Research Centre (CNIO) in Madrid, where he has been employed since 2002. Professor Montoya was identified as the absolute top candidate to lead a new CPR Program focused on structural analysis of medically relevant proteins. Professor Montoya studied chemistry and obtained his PhD at the University of Zaragoza. He moved for his first postdoctoral position to the Max Planck Institute for Biophysics in Frankfurt and from there to the European Molecular Biology Laboratory (EMBL) in Heidelberg. After finishing his postdoctorate, Professor Montoya pursued an independent group leader career as head of the Macromolecular Crystallography Group in the Structural Biology and Biocomputing Program at the Spanish National Cancer Center (CNIO) in Madrid.

The career of Professor Montoya is marked by impressive achievements in elucidating atomic structure of complex protein machines governing vital physiological processes such as unwinding DNA during genome replication and chromosome segregation during mitosis. Professor Montoya's research interest perfectly integrates with the biomedical focus of CPR on disease mechanisms associated with malfunctions of protein pathways involved in genome integrity maintenance. Equally important, his expertise in X-ray crystallography and electron microscopy significantly expands the protein technology portfolio at CPR and further increases our outreach to collaborators at the Faculty of Health and Medical Sciences and beyond. The new Protein Structure Program will leverage the state-of-the-art protein expression technologies available at CPR. Professor Montoya will therefore integrate and develop our Protein Production Facility in the new program. The new program on protein structure perfectly complements the already established CPR programs and completes our portfolio of cutting edge protein-based technologies.



### Statement by the Chairman of the Scientific Advisory Board

"The SAB always enjoys its visit to Copenhagen and CPR. Both for the quality of the science, but also for the relaxed and creative atmosphere. This year was no exception. Jiri presented a coherent and ambitious plan for the future, and we look forward to following its development over the coming years. Although I have been on the board since the beginning of CPR, this is my first year as chair, and I would like to take the chance to thank Chris Austin for all his efforts to mold the SAB into a collaborative and functioning unit"

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Anthony Hyman Chairman of the SAB 2013 Director of the Max Planck Institute of Molecular Cell Biology and Genetics, Dresden (Germany) SAB photographed with CPR management, the Dean of the Faculty of Health and Medical Sciences and CPR group leaders at the board meeting in May 2013.

From the left (row by row): Chunaram Choudhary (CPR), André Nussenzwieg (SAB), Niels Mailand (CPR), Ivan Dikic (SAB), Poul Nissen (SAB), Pernille Rørth (SAB), Jakob Nilsson (CPR), Anthony Hyman (SAB), Lars Juhl Jensen (CPR), Angus Lamond (SAB), Ulla Wewer (Dean), Søren Brunak (CPR), Michael L. Nielsen (CPR), Jeremy A. Daniel (CPR), Jesper V. Olsen (CPR), Matthias Mann (CPR), Jiri Lukas (CPR), Peter Dyrsting (CPR), Mats Wikström (CPR) & Amilcar Flores-Morales (CPR).

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### **NNF Center for Protein Research Organizational Chart**



CPR leadership, research programs and technological facilities (in brackets: Number of employees in December 2013).



### **Center Management**

The Novo Nordisk Foundation Center for Protein Research is a research center at the Faculty of Health and Medical Sciences at the University of Copenhagen.

CPR is managed by an executive management team consisting of:

- Ulla Wewer, Dean of the Faculty of Health and Medical Sciences
- · Jiri Lukas, Executive Director, Program Director
- Jesper Velgaard Olsen, Vice Director
- Matthias Mann, Program Director
- Søren Brunak, Program Director
- Guillermo Montoya, newly appointed Research Director (from January 2014)
- Peter Dyrsting, Head of Administration

The team works as a three-tier management based on frequent interactions: (1) the dean, the executive director and the vice director typically meet twice a month to discuss strategic matters, (2) the program directors meet once a month to take care of the scientific development, and (3) the executive director, the vice director and the head of administration meet every week. In addition, group leader meetings take place 6-8 times a year.

The CPR group leaders include:

**Disease Mechanisms Program** 

- Professor Jiri Lukas (Chromosome Stability and Dynamics)
- Associate Professor Jeremy A. Daniel (Chromatin Structure and Function)
- Associate Professor Jakob Nilsson (Mitotic Mechanisms and Regulation)
- Professor Amilcar Flores-Morales (Molecular Endocrinology)
- Professor Niels Mailand (Ubiquitin Signaling)

**Disease Systems Biology Program** 

- Professor Søren Brunak (Translational Disease Systems Biology)
- Professor Lars Juhl Jensen (Cellular Network Biology)

#### **Proteomics Program**

- Professor Jesper Velgaard Olsen (Mass Spectrometry for Quantitative Proteomics)
- Professor Chunaram Choudhary (Proteomics and Cell Signaling)
- Associate Professor Michael Lund Nielsen (Proteomics Technology Development and Application)

#### Protein Structure Program (from January 2014)

- Professor Guillermo Montoya
- Associate Professor Mats Wikström



### **Scientific Advisory Board**

A very active Scientific Advisory Board is an important asset of an ambitious research center. We are fortunate to have a group of prominent and well-known scientists on our Scientific Advisory Board, all of them genuine scientific authorities in their respective scientific fields.

The board provides advice on the strategic direction of the Center as well as on the progress and development of the scientific program. The board also assists in the establishment of external collaborations.

Board members are invited by the Center's executive management, each member providing expertise and experience within one or more of the Center's scientific fields. Presently, the board consists of 7 members:

Anthony Hyman (chair), Group Leader and Director of the Max Planck Institute of Molecular Cell Biology and Genetics, Dresden (Germany): Cell cycle control, high-throughput screens. Ivan Dikic, Director of the Institute of Biochemistry, Goethe University, Frankfurt and Scientific Director of the FMLS/Frankfurt Institute for Molecular Life Sciences (Germany): Ubiquitin signaling.

Angus Lamond, Professor of Biochemistry, Wellcome Trust Centre for Gene Regulation and Expression, College of Life Sciences, University of Dundee (UK): Proteomics, advanced imaging. Poul Nissen, Principal Investigator at Department of Molecular Biology, Centre for Structural Biology, University of Aarhus (DK): Structural biology. Pernille Rørth, Deputy Director, Institute of Molec ular and Cell Biology, A-STAR (Singapore): Cell motility, developmental biology. Torben Falck Ørntoft, Head of Department of Molecular Medicine, Aarhus University Hospital, Skejby (DK): Translational cancer research. André Nussenzweig, Chief of Laboratory of Genome Integrity, NCI/NIH, Center for Cancer Research (US): DNA damage response, mouse models of genome instability disorders.

Sadly, Tony Pawson, who has been part of the CPR Scientific Advisory Board since its beginning, passed away in the summer of 2013. Chris Austin resigned from his position as Scientific Advisory Board chair and was succeeded by Anthony Hyman. In 2013, the Scientific Advisory Board welcomed Angus Lamond from the University of Dundee and André Nussenzweig from NCI/NIH.



Tony Hyman (Chair), Poul Nissen, Pernille Rørth, Torben Falck Ørntoft, Ivan Dikic and André Nussenzweig.

### **CPR** Timeline



Major events in CPR's history, from the point of the Novo Nordisk Foundation donation in April 2007, to the end of 2013.



### **Disease Mechanisms Program**

The Disease Mechanisms Program consists of five research groups that explore protein pathways spanning ubiquitin signaling (Niels Mailand), mechanisms of chromosome segregation (Jakob Nilsson), mouse models for protein guardians of the genome (Jeremy A. Daniel), pathways involved in pathogenesis of endocrine tumors (Amilcar Flores-Morales), and advanced imaging of protein regulators of cell cycle and DNA damage responses (Jiri Lukas). The program complements the CPR setup by generating 'physiological endpoints' of protein research and providing mechanistic insight into medically relevant protein modifications and signaling pathways.

Equally important, the program contributes to the CPR protein technology pipeline through its world-leading expertise in automated protein imaging and high-content image-based screens to identify and functionally characterize new proteins in their natural environment.

This technology has been key to many of our successes and also a major incentive to launch a Protein Imaging Facility together with our colleagues from the neighboring DanStem Center. We were very fortunate to recruit Jutta Bulkescher, formerly senior microscopy technician at the Advanced Light Microscopy Facility at EMBL, to support virtually all CPR projects that involve protein imaging. This initiative has been a great success and has potential to further increase collaborative efforts across all CPR Programs and to spark new interactions within the NNF Center Cluster.

### Chromosome Stability & Dynamics/Lukas Group Group Leader: Professor Jiri Lukas

The Chromosome Stability and Dynamics Group moved to CPR in June 2012, and 2013 was thus the first full year spent at CPR. The main research focus of the group remains on development and application of advanced protein imaging technologies and high-content screens to study the structure, dynamics and posttranslational modifications of chromatin exposed to various forms of stress. In 2013, the group was expanded with two post-docs from the University of Zurich. With these two additions, the Lukas group is fully staffed and comprises 1 professor, 1 associate professor, 4 postdocs, 2 PhD students, 2 lab technicians and 1 laboratory assistant.

#### **Research aim**

Most current projects in the Lukas group revolve around a distinct nuclear sub-compartment (so called 'repair focus') generated at the sites of damaged chromosomes and attracting an exquisitely complex, hierarchical, and dynamic congregation of DNA- and histone-associated proteins and protein modifications. Highly coordinated spatial and temporal action of these so-called 'genome caretakers' is crucial to prevent chromosomal aberrations. We are particularly interested in cellular responses to stress induced by DNA replication because of its broad relevance for the etiology and treatment of a broad range of diseases characterized by unstable genomes.

#### **Key achievements**

In 2013, the Lukas lab investigated diverse aspects of protein-based genome surveillance and published in total 7 primary research articles (*3, 9, 24, 38, 82, 86, 89*) and 3 review/opinion articles (*2, 4, 50*). The two key discoveries can be summarized as follows:

We identified the SAFB1 chromatin architecture protein as a sensor of Poly(ADP-ribosyl)ation at the sites of DNA damage, where it regulates spreading of downstream posttranslational modifications (PTMs) including ATM/ATR-mediated histone phosphorylation. The key person behind this project was Matthias Altmeyer, the first author of a research article published in Molecular Cell (3).

Apart from launching a precedent for the direct involvement of chromatin architecture proteins in determining the magnitude of the DNA damage-induced PTMs, this work has a high potential to help us understand the molecular underpinnings of Poly(ADP-ribosyl)-mediated protein accumulation at damaged chromosomes. Matthias Altmeyer is again coordinating this work both in the lab and as a broader inter-CPR collaboration including the Proteomics Program (Michael Lund Nielsen group) and the Protein Science Facility (Werner Streicher, Irina Pozdnyakova).



The other key discovery was made by Luis Toledo, the first author of a study published in Cell (82), which showed that replicating genomes are guarded against irreversible damage by the Replication Protein A (RPA) complex. Most significantly, this study shows that human cells have only limited supply of RPA, which can be further reduced after oncogenic transformation. While working on this project, Luis developed a new protein imaging technique (Quantitative Image-Based Cytometry, QIBC), which has potential to spread from a laboratory discovery tool to the clinic as a means to asses sensitivity of cancer cells to chemotherapy. Importantly, this project also sparked internal CPR collaborations, which already involve several groups from different programs including Niels Mailand (Disease Mechanisms), Jesper Velgaard Olsen (Proteomics), and Jeremy A. Daniel (Disease Mechanisms).

#### Impact and outreach

In 2013, Jiri Lukas was invited to speak at three international conferences: Chromatin, Replication and Chromosomal Stability; Abcam (Copenhagen, June 17-19), Eukaryotic DNA Replication and Genome Maintenance, Cold Spring Harbor (US, September 9-13), Chromosome Architecture in Human Cancers, National Institute of Health (Rockville US, November 12-13).

Jiri Lukas was awarded DKK 3.5 M from the Danish Cancer Society for a project entitled 'Search for novel cancer genes in the uncharacterized fraction of the human genome'. Postdoc Matthias Altmeyer was awarded DKK 2.5 M from the Danish Council for Independent Research (Medical Sciences) and additional DKK 0.5M as a Sapere Aude: DFF Graduate Talent award for a project entitled 'Identification and characterization of concealed regulators of genome maintenance pathways'.

**Lukas Group:** Group leader Jiri Lukas, associate professor Claudia Lukas, postdocs Matthias Altmeyer, Luis Toledo, Dorthe Larsen & Kai Neelsen, PhD students Thorkell Gudjónsson & Ronni Sølvhøj Pedersen, technicians Merete Grøfte & Maj-Britt Rask and laboratory assistant Andreas Willems. Refer to the 'Staff' section for a full list of group members.



#### **External collaborators**

Jan Ellenberg, Rainer Pepperkok, Beate Neumann and Jean-Karim Heriche (Advanced Light Microscopy Facility, EMBL, Heidelberg, Germany) on high-content imaging and siRNA screens.

**Ian Hickson** (Center for Healthy Aging, Faculty of Health and Medical Sciences, UCPH) on screens for DNA repair proteins.

**Jiri Bartek** (Danish Cancer Society) on protein pathways subverted in cancer.

**André Nussenzweig** (NCI/NIH, Bethesda, US) on the function of RNF168 ubiquitin ligase.

**Oskar Fernandez-Capetillo** (CNIO, Madrid, Spain) on functional characterization of the ATR/CHK1 signaling pathway.

**Kirsten Grønbæk** (Department of Hematology, Rigshospitalet, DK) on improving stratification of patients with hematological malignancies based on RPA imaging assays developed in our group.

#### Internal collaborators

**Michael Lund Nielsen** (Proteomics Program) on the role of protein modification by Poly(ADP-ribosyl)ation in genotoxic stress responses.

Jesper Velgaard Olsen (Proteomics Program) on ATR phospho-proteome and its function during replicative stress.

**Niels Mailand** (Disease Mechanisms Program) on structural and functional dissection of ubiquitin-regulated surveillance pathways.

**Jeremy A. Daniel** (Disease Mechanisms Program) on protein pathways guarding mammalian genomes against replication stress.

**Werner Streicher** and **Irina Pozdnyakova** (Disease Mechanisms Program) on the mechanism of Poly(ADP-ribose)-mediated protein accumulation at damaged chromosomes.

### Chromatin Structure & Function/Daniel Group Group Leader: Associate Professor Jeremy Austin Daniel

The Chromatin Structure and Function Group was established in January 2012 and has just finished its second year at CPR. The group is currently composed of 3 postdocs, 1 master student, 1 laboratory technician and 1 student assistant.

The mission of the Daniel lab is to investigate basic mechanisms for how dynamic chromatin environments impact the stability of our genomes. DNA double-strand breaks (DSBs) can be caused by exogenous damage, collapsed replication forks, and can also transiently occur during normal physiology as part of programmed DNA rearrangements in lymphocytes called V(D)J recombination and class-switch recombination. Once damaged, a DNA lesion such as a DSB must first be made accessible by changes in chromatin structure to allow for subsequent DNA repair to occur. A better physiological understanding of protein pathways and signaling involved in DNA repair within the context of chromatin holds important implications for healthy aging and disease management, including sensitizing cancer cells to therapeutic agents.

The approach of the Daniel lab is to utilize mice as a physiological model together with a combination of biochemistry, flow cytometry, genomics, and proteomics for investigating how protein complexes that modify or associate with chromatin regulate genomic stability and prevent lymphoid malignancy. Proteomic screens for chromatin-interacting factors and chromatin modifying complexes are being carried out in combination with targeted gene inactivation in mice.

#### **Research aim**

The aim of the research in the Daniel lab during 2013 was to continue developing the research projects initiated in its first year and initiate additional projects with newly hired personnel. Specifically, the group aimed to develop a project investigating the mechanism and specificity for how the PTIP-associated histone H3K4 methyltransferase complex functions in B lymphocytes. The group also aimed to initiate research projects that investigate the role of acetyl-transferases in B lymphocytes and identify functional proteins at DNA breaks with label-free quantitative proteomics in mouse tissues.

#### Key achievements

In 2013, the Daniel lab published 3 primary research articles as a co-author (*15, 48, 57*) and 1 review article (*20*). The group also made progress on multiple projects in the lab. To investigate the mechanism and specificity of PTIP complex function in B lymphocytes, the group has obtained preliminary evidence for a single amino acid that is responsible for the function of PTIP in IgH class-switch recombination; for the region of PTIP that interacts with the MLL-like histone H3K4 methyltransferase complex and the significance of this interaction for IgH class-switch recombination; and has targeted embryonic stem cells for generation of a novel knock-in mouse model.

#### **External collaborators**

**Sharon Y. Dent** (University of Texas MD Anderson Cancer Center, US) to investigate the role of acetyltransferases in B lymphocytes.

**André Nussenzweig** (NCI/NIH, US) to investigate the mechanism and specificity of PTIP complex function in B lymphocytes.

**Kai Ge** (NIDDK/NIH, US) to investigate the mechanism and specificity of PTIP complex function in B lymphocytes.

#### Internal collaborators

**Michael Lund Nielsen** (Proteomics Program) to identify functional proteins at DNA breaks with quantitative proteomics in primary lymphocytes.

**Chunaram Choudhary** (Proteomics Program) to investigate the mechanism and specificity of PTIP complex function in B lymphocytes.

**Niels Mailand** (Disease Mechanisms Program) to investigate the biological function of novel DNA damage response factors with murine gene inactivation.

Jiri Lukas (Disease Mechanisms Program) to investigate the role of RPA in modulating cancer with mouse models.

To investigate the role of acetyltransferases in B lymphocytes, the group has obtained preliminary evidence for an acetyltransferase that promotes IgH class-switching and is currently developing unique genetic tools to explore this further *in vivo*.

To identify functional proteins at DNA breaks with quantitative proteomics, the group has developed an experimental workflow using primary murine lymphocytes for which they will build upon in the coming years.

**Daniel Group**: Group leader Jeremy A. Daniel, postdocs Linda Marie Starnes & Andreas Mund, master student Laura Maarit Pikkupeura, technician Rebeca Soria Romero and research assistant Cody Colon-Berezin. Refer to the 'Staff' section for a full list of group members.

#### Impact and outreach

A literature review article from the group published in Molecular Cell (20) was aimed to begin establishing in the DNA damage response field that the Daniel lab is an independent research group working in Copenhagen, Denmark.

Members of the group participated in international conferences including a Keystone Symposium in Banff, Canada and an Abcam meeting in Copenhagen. The group leader was an invited speaker to the Department of Biology at the University of Copenhagen and was also invited to speak as a member of the scientific community at the Copenhagen Science City outreach dinner event and at the Copenhagen Bioscience Conference on Genomics in Metabolism. Members of the group served as peer-reviewers for multiple manuscripts and were either lecturers or instructors for two different immunology courses teaching bachelors and master students at the University of Copenhagen.



### Mitotic Mechanisms & Regulation/Nilsson Group Group Leader: Associate Professor Jakob Nilsson

The Mitotic Mechanisms and Regulation Group joined CPR in 2011. The group is headed by Associate Professor Jakob Nilsson and is currently composed of 5 postdocs, 3 PhD students and 1 laboratory assistant.

The Nilsson lab focuses on mechanistic aspects of the chromosome segregation process during cell division. Failure in proper chromosome segregation is detrimental to the cell and is the underlying cause of a number of human diseases, including cancer. The detailed understanding of the proteins regulating cell division is therefore of importance as it constitutes the foundation for developing strategies to target them. Indeed a number of novel compounds inhibiting proteins involved in chromosome segregation are in clinical trials aimed at treating certain types of cancers.

A major focus of the Nilsson lab is on the so-called Spindle Assembly Checkpoint (SAC) that delays cell division until all chromosomes have aligned on the metaphase plate. The activity of SAC is controlled by a large protein structure on chromosomes referred to as the kinetochore. The kinetochore attaches the chromosomes to the mitotic spindle and this is required for the movement of chromosomes into the daughter cells. In the presence of unattached kinetochores, all SAC proteins accumulate at this structure and this is required for generating an inhibitory signal.

#### **Research aim**

The aim of the Nilsson lab in 2013 was 1) to deepen our understanding of how SAC proteins are recruited to kinetochores and how this is regulated, 2) understanding how the inhibitory SAC signal prevents chromosome segregation, and 3) mapping of post-translational modifications and their dynamics during cell division.

#### Key achievements

The Nilsson group has made extensive progress in understanding how a number of SAC proteins are recruited specifically to unattached kinetochores and how this is regulated. We focused on the SAC complexes Bub1-Bub3 and BubR1-Bub3 and found that they have multiple independent binding sites on the kinetochore protein KNL1 (91).



Bub3 was able to bind directly to phosphorylated MELT repeats in KNL1 and this interaction was negatively regulated by the PP1 phosphatase during an active SAC. KNL1 contains 12 MELT repeats, but surprisingly we found that only 4 where required for proper chromosome segregation and for a fully functional SAC. Since both Bub1 and BubR1 depend on Bub3 for interaction with KNL1, the next important step will be to understand how these SAC complexes regulate each other as BubR1 depends on Bub1 for kinetochore recruitment.

Mitotic progression is driven by the Anaphase Promoting Complex (APC/C), a large ubiquitin ligase that targets key mitotic proteins for degradation. The APC/C is activated by the protein Cdc20 during mitosis and the SAC prevents APC/C activity by inhibiting Cdc20. Cdc20 is inhibited by the direct binding of the two SAC proteins BubR1 and Mad2 forming the mitotic checkpoint complex (MCC). We have made progress in understanding how the MCC prevents mitotic progression by showing that the stable binding of the MCC to the APC/C is required for proper SAC function (Hein, J & Nilsson, J: 'Stable MCC binding to the APC/C is required for a functional Spindle Assembly Checkpoint' *EMBO reports, in press*). As only a fraction of APC/C is bound to the MCC, this suggests that only a small fraction of APC/C is active during mitosis and understanding the nature of this pool is an important future research direction.

#### Impact and outreach

The mechanistic insight we achieve through our work provides an important foundation for future research and is important for developing approaches to target pathways that are deregulated in human diseases. In 2013, the group published 4 primary research papers as first and last authors (44, 70, 91, 92). The work on KNL1 (91) was highlighted by Journal of Cell Science and a dispatch in Current Biology.

Jakob Nilsson was awarded a grant from the Danish Cancer Society (DKK 1.2 M) to explore the role of phosphatases during mitosis and postdoc Garry G. Sedgwick was awarded a grant from Danish Council for Independent Research (Medical Sciences) (DKK 2.1 M) to explore novel regulators of cell division.

Group members have actively participated in international meetings and presented posters as well as oral presentations. Jakob Nilsson was invited speaker to "Dynamic Kinetochore Workshop 2013" in Porto as well as to the Department of Genetics, Cambridge. Additionally, Jakob Nilsson is on the MoMed PhD school advisory board and heads the Nordforsk Mitosis Network.



#### **External collaborators**

**Victor M. Bolanos Garcia** (Oxford Brookes University, Oxford, UK) on structural investigations of mitotic regulators.

**Jennifer DeLuca** (Colorado State University, US) on regulation of kinetochore activity.

#### Internal collaborators

**Amilcar Flores-Morales** (Disease Mechanisms Program) on investigating mitotic functions of proteins involved in prostate cancer.

Jesper Velgaard Olsen (Proteomics Program) on identifying novel interactors of phosphatases during mitosis.

**Michael Lund Nielsen** (Proteomics Program) on improving methods for detecting transient protein interactions in cells.

Chunaram Choudhary (Proteomics Program) on regulation of APC/C activity.

**Mats Wikström** and **Werner Streicher** (Protein Science Facility) on the role of PTMs in regulating protein-protein affinities.

**Nilsson Group:** Group leader Jakob Nilsson, postdocs Daniel G. Hayward, Thomas Kruse, Garry G. Sedgwick, Gang Zhang & Tiziana Lischetti and PhD students Julie Schou, Marie Sofie Yoo Larsen & Jamin Hein. Refer to the 'Staff' section for a full list of group members.

### Molecular Endocrinology/Flores-Morales Group Group Leader: Professor Amilcar Flores-Morales

The Molecular Endocrinology Group was established in the autumn of 2009, is led by Professor Amilcar Flores-Morales and includes 3 postdocs, 1 PhD student and 1 BSc student.

Prostate cancer is the most commonly diagnosed cancer among men and one of the main causes of cancer-related deaths in the western world. Patients with prostate cancer die because of our inability to treat advanced metastatic tumors. The overall aim of the Flores-Morales lab is to identify novel strategies for the prevention and treatment of advanced prostate cancer. To this end, we make extensive use of high-throughput quantitative analyses, including gene expression and proteomics profiling to analyze human tissue biopsies as well as animal and cell models in order to outline clinically relevant signaling pathways involved in prostate cancer progression. We are especially interested in understanding cross-talk between protein-modifying enzymes, such as kinases and ubiquitin ligases, and the transcriptional actions of the androgen receptor, a key driver of prostate tumorigenesis at all stages.

#### **Research aim**

In 2013, the Flores-Morales lab had two key aims. The first was to test the function of two androgen receptor regulated proteins and potential tumor suppressors, SOCS2 and REST, in prostate carcinogenesis.

The second was to implement a novel strategy for the quantitative analysis of the proteome of prostate biopsies and apply it to the study of mouse models of prostate cancer progression and clinical samples.



#### **Key achievements**

The work of the Flores-Morales lab in 2013 has led to the identification of several novel regulators of prostate progression as well as a clinically validated biomarker of disease progression. We demonstrated that the transcriptional repressor REST is controlled by the androgen receptor in prostate cancer cells and contribute to the differentiation of prostate epithelium (78). Importantly, the analysis of REST expression in a cohort of prostatectomy samples demonstrated that low REST levels identify a subset of localized tumors with increased aggressiveness. Patients bearing tumors with the lowest REST levels have 10 times increased risk of disease recurrence after prostatectomy as compared to those with the highest levels. Multivariate analysis demonstrated that REST is an independent indicator of disease aggressiveness not influenced by tumor histology and as such may constitute a novel biomarker to separate patients in need of active treatment from those harboring indolent disease. (78)



#### Internal collaborators

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**Michael Lund Nielsen** (Proteomics Program) on identification of ubiquitination targets for the SOCS family of ubiquitin ligases.

**Jesper Velgaard Olsen** (Proteomics Program) on phosphorylation dependent mechanisms regulating castration resistant prostate cancer growth.

**Jakob Nilsson** (Disease Mechanisms Program) on investigating mitotic functions of proteins involved in prostate cancer. In another publication, we demonstrated that SOCS2 has tumor suppressor functions in prostate cancer. SOCS2 is transcriptionally stimulated by androgens in prostate cancer cells and subsequently inhibits activity of JAK2 and Src kinases. In situations associated with reduced testosterone levels such as what arises upon androgen ablation therapy of prostate cancer patients, SOCS2 expression is reduced, allowing for the activation of kinase driven mechanisms to maintain tumor growth. Therefore, we proposed that reduced SOCS2 expression in castration resistant tumors may serve to identify individuals susceptible to treatment with JAK2 and Src kinase inhibitors (32). We are currently investigating the basis for substrate recognition by the members of the SOCS family of ubiquitin ligases to gain understanding on their tissue specific actions (5, 25).

#### Impact and outreach

The work of the Flores-Morales lab has great clinical relevance. In 2013, this led to the identification of REST, a possible new biomarker to separate patients in need of active treatment from those harboring indolent disease. A research team coordinated by Amilcar Flores-Morales received the Movember Danish Prostate Cancer Translational Research Award of DKK 2.5 M for the project 'Mass spectrometry based proteome profiling for precision medicine in prostate cancer'. The team comprises scientists from the Olsen group (Proteomics Program), the CPH Prostate Cancer Center/Rigshospitalet and Center for Biological Sequence Analysis at DTU. Flores-Morales also received DKK 1 M from the Novo Nordisk Foundation for the project 'Tumor Suppressor functions of SOCS proteins in prostate cancer'.

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#### External collaborators

**Anders Bergh** (University of Umeå, Umeå, SE) on proteomic profiling of prostate cancer bone metastases.

**Anders Bjartell** (University of Lund, Malmö, SE) on characterization and validation of novel biomarkers in prostate cancer.

**Colin Collins** (University of British Columbia, Vancouver, Canada) on proteomic based decision making for the treatment of castration resistant prostate cancer.

**Yuanjie Niu** (Tianjin Medical university, Tianjin, China) on characterization of Hippo signaling pathways in advanced prostate cancer.

**Matthias Mann** (Max Planck Institute for Biochemistry, Munich, Germany) on clinical proteomics.

**Tamar Geiger** (University of Tel Aviv, Tel Aviv, Israel) on clinical proteomics.

**Leandro Fernandez-Perez** (University of Las Palmas GC, Las Palmas de Gran Canaria, Spain) on tumor suppressor functions of SOCS2 in prostate cancer.

**Gunnar Norstedt** (Karolinska Institutet, Stockholm, SE) on inhibition of JAK/STAT signaling for the treatment of castration resistant prostate cancer.

### **Ubiquitin Signaling/Mailand Group** Group Leader: Professor Niels Mailand

The Ubiquitin Signaling Group was established in the autumn of 2009. In 2013, a number of new PhD students and postdocs, mostly international, were recruited to the lab. As of the end of the year, the group has 14 lab members (1 professor, 2 associate professors, 4 postdocs, 6 PhD students, and 1 MSc student).

The key focus of the group is to understand how signaling mediated by ubiquitin and ubiquitin-like modifier proteins (UBLs) promote cellular responses to DNA damage and maintenance of genome stability in human cells. Our principal strategy is to utilize the combined powers of unbiased proteome- and genome-wide approaches and focused cell-based studies in a multipronged strategy towards discovery and detailed functional characterization of new factors and ubiquitin-dependent processes that protect the integrity of the genome following DNA damage. Through fruitful in-house collaboration with technology-oriented groups at CPR, we are performing proteomics- and microscopy-based screens for new factors and signaling processes in the DNA damage response. Individual hits from screens are subsequently subjected to in-depth mechanistic studies of their functional and biological significance, using a range of focused cell biology-, biochemistry-, and imaging-based methods in which the group has strong expertise.

#### **Research aim**

Most of the current research endeavours and directions of the lab fit broadly into four complementary and interconnected themes:

- Exploration of the DNA damage-regulated ubiquitylome.
- Ubiquitin-dependent orchestration of protein recruitment to sites of DNA damage.
- Characterization of new factors in the DNA damage response.
- Biological function of poorly characterized UBLs.

#### Key achievements

The group continued to employ proteomics-based methods to identify ubiquitin-dependent signaling processes regulated by DNA damage and related insults. The unique insights gained from these studies enabled us to discover and mechanistically characterize an all-new ubiquitin-regulated cellular stress response that causes dramatic remodeling of cellular structures known as centriolar satellites (*85*). We are now aiming to take the approaches for mapping DNA damage-regulated ubiquitylation processes in an unbiased manner to a further advanced level, by



**Mailand Group:** Group leader Niels Mailand, associate professor Simon Bekker-Jensen & Tina Thorslund, postdocs Godelieve Smeenk, Sara Lund Poulsen, Petra Schwertman, Ian Gibbs-Seymour & Yasuyoshi Oka, PhD students, Maxim Tollenaere, Rebecca Kring Hansen & Saskia Hoffmann, research assistants Anita Ripplinger & Peter Haahr, Louise Aagaard Nilausen, Stine Emilie Weis-Banke. Refer to the 'Staff' section for a full list of group members.

quantitatively monitoring ubiquitin-dependent signaling processes that occur locally at damaged DNA. We anticipate that this will enable us to obtain unprecedented new insights into the multi-faceted roles of ubiquitin in protecting genome stability following DNA damage.

A new major focus of the lab initiated in 2013 is the indepth characterization of a range of new factors in the DNA damage response, identified in collaboration with Matthias Mann's lab in Munich. These studies are yielding exciting and important new insights into several aspects of the cellular DNA damage response, such as the repair of DNA double-strand breaks (DSBs), and we anticipate that further dedicated studies of these factors and the processes they regulate will remain a key priority for the lab in the years to come.

Understanding the signaling mechanisms underlying protein recruitment to sites of DNA damage, particularly DSBs, has been a long-standing interest of the lab. Using affinity reagents highly specific for isolation of K63-linked ubiquitin chains, which have a critical, yet still unresolved, role in orchestrating DSB repair, we have now identified a potential key substrate of K63linked ubiquitylation in this response. The mechanistic basis of DNA damage-dependent ubiquitylation of this factor and its ramifications for efficient DSB repair is now under intense scrutiny in the lab.



Recently, we have become interested in how ubiquitin regulates protein recruitment to the replication machinery upon encounters with damaged DNA (53). Microscopy-based siRNA screens for ubiquitin-signaling factors that regulate recruitment of translesion DNA synthesis (TLS) polymerases and related factors to PCNA at the replication fork have provided new leads as to the poorly defined regulatory framework underpinning TLS, the mechanistic basis of which we are now investigating in detail.

Finally, we discovered a new SUMO-targeted ubiquitin ligase, RNF111 that functions in the DNA damage response and other cellular processes (*66*) and we are finalizing functional studies of several poorly characterized UBLs in human cells, including UBL5.

#### Impact and outreach

The group published 9 papers in 2013 (*19, 26, 41, 53, 58, 66, 71, 82, 85, 89*), most of which were in high-impact biological journals, including Cell, Molecular Cell, Journal of Cell Biology, EMBO Journal, and Nature Reviews Molecular Cell Biology. One paper (in EMBO J, *85*) was highlighted in a News&Views article in the same issue; one (in Nature Rev Mol Cell Biol, *53*) was highlighted on the cover.

The group extended its highly successful track record in receiving grants and recognition in the form of prestigious awards. Niels Mailand was awarded a highly competitive, 5-year Consolidator Grant ( $\in$  2 M) from the European Research Council (ERC). He also received the Silver Medal from the Royal Danish Academy of Sciences and Letters, and was selected as an EMBO Young Investigator.

#### **External collaborators**

**Matthias Mann** (Max Planck Institute of Biochemistry, Martinsried, Germany) on Proteomics-based discovery of new components of the DNA damage response.

**Ivan Dikic** (University of Frankfurt, Germany) on Ubiquitin signaling in autophagy.

**Wim Vermeulen** (ErasmusMC, Rotterdam, The Netherlands) on Ubiquitylation in nucleotide excision repair.

**Roger Pocock** (BRIC, UCPH, DK) on studies of orthologues of human DNA damage response factors in C. elegans

#### Internal collaborators

**Chunaram Choudhary** (Proteomics Program) on Proteomics-based exploration of the ubiquitin system.

**Jiri Lukas** (Disease Mechanisms Program) on advanced imaging-based cell analysis.

**Jeremy A. Daniel** (Disease Mechanisms Program) on mouse knockout models of new DNA damage response factors.

### Protein Science Facility/Wikström Group Group Leader: Associate Professor Mats Wikström

The Protein Science Facility was established in August 2012 as part of the CPR midterm transition. It is led by Associate Professor Mats Wikström and represents a fusion of two units: (1) The Protein Production Facility and (2) the Protein Function and Interactions group (PFI).

The Protein Production Facility is responsible for protein production of recombinant proteins in prokaryotic and eukaryotic hosts, generating protein samples for internal CPR projects and for selected strategic partners and collaborators. In addition, the facility has expertise in protein purification techniques and biophysical characterization of the produced target proteins. The Protein Production Facility currently consists of 2 team leaders (academic coordinators), 7 laboratory technicians, 1 academic research technician, 1 laboratory assistant and is structured into four teams; the *Prokaryotic* and *Eukaryotic Teams*, responsible for all steps from sub-cloning of target proteins and recombinant expression to purification of target proteins using affinity tags. The *Protein Purification Team* provides additional support in terms of classical chromatography. Finally, the *Biophysics Team* is responsible for quality control of the recombinant proteins using biophysical techniques.

The Protein Function and Interactions group is an independent research group focused on understanding molecular recognition with particular emphasis on protein-protein interactions. The group has two PhD students and three postdoctoral fellows. This group has, through their internal projects, established strong platforms within biophysics and structural biology.

#### **Research aim**

The research projects within Protein Function and Interactions are focused on the study of the structure, dynamics and interactions of biological macromolecules. The primary objective is to understand molecular recognition in protein-protein interactions using both biophysical methods and structural biology approaches (NMR spectroscopy and X-ray crystallography).

#### Key achievements

The current projects are within the following areas:

- Structure and dynamics of SUMO Interacting Motifs
   Structure and function for virulence determinants
- of the bacterial pathogen *Streptococcus pyogenes*Biophysical and structural studies of the interaction matrix of insulin-like growth factor-binding proteins (IGFBPs)
- Biophysical and structural characterization of PICH



Our group has been collaborating with the Mailand group on the identification of a novel SUMO-binding ZZ Zinc finger motif in HERC2 (Danielsen et al., 2012, *JCB* 197:179). By the application of hetero-nuclear NMR we have now identified the surfaces involved in the interaction between a ZZ domain and SUMO1. Based on this data, we have been able to generate a model for the structure of the complex using <sup>15</sup>N chemical perturbation data in combination with RDCs (residual dipolar couplings). Interestingly, the primary binding site on SUMO for the ZZ domain is distinctly different from the previously defined binding-site for classical Sumo Interacting Motifs

Streptococcus pyogenes is a significant bacterial pathogen in the human population. The importance of virulence factors for the survival and colonization of *S. pyogenes* is well established, and many of these factors are exposed to the extracellular environment enabling bacterial interactions with the host. We have solved the three-dimensional structure of one of the



most abundant virulent proteins. Its three-dimensional structure was determined (see figure) and showed a novel tetrameric organization composed of four helix-loop-helix motifs. Affinity pull-down mass spectrometry analysis using human plasma demonstrated that the protein interacts with histidine-rich glycoprotein (HRG), and the name sHIP (streptococcal Histidine-rich glycoprotein Interacting Protein) is therefore proposed. HRG has antibacterial activity, and when challenged by HRG, sHIP was found to rescue *S. pyogenes* bacteria. This and the finding that patients with invasive *S. pyogenes* infection respond with antibody production against sHIP, implies a role for the protein in *S. pyogenes* pathogenesis.

#### Impact and outreach

One major objective for the Protein Science Facility is the responsibility for production of recombinant proteins in prokaryotic and eukaryotic hosts, generating protein samples needed for internal CPR projects and for selected strategic partners and collaborators. The Protein Science Facility has been involved in the generation of a number of protein samples during 2013, resulting in a number of publications (5, 7, 11, 27, 44, 54, 59, 66). The unit has been particularly successful in developing its human HEK293 platform with emphasis on secreted human proteins.

The research part (PFI) has established strong platforms in biophysical and structural studies of biological macromolecules. During 2013, we have identified the binding site on SUMO for the novel SUMO-binding ZZ-domain including the generation of a model for the complex. In addition, we have identified a novel extracellular protein, sHIP, from the human pathogen *Streptococcus pyogenes* that binds and inhibits the antibacterial activity of human histidine-rich glycoprotein. The three-dimensional structure of sHIP represents the first protein structure determined at the CPR (PDB accession # 4MER).

#### **External collaborators**

Lars Björck & Johan Malmström (Department of Clinical Sciences, Faculty of Medicine, Lund University, Lund, and Lund University Hospital, Lund, Sweden) on mechanistic studies on a set of novel virulence determinants from the bacterial pathogen Streptococcus pyogenes.

**Michael Gerstenberg** (Novo Nordisk, Copenhagen, DK) and **Ming Wei Wang** (National Center for Drug Screening, Shanghai, China) on the interaction matrix for insulin-like growth factor binding proteins. **Ian Hickson** (Center for Healthy Aging, University of Copenhagen, DK) on protein-protein interactions in

genome stability.

**Facing page, Protein Science Facility:** Group leader Mats Wikström, academic coordinators Werner Streicher & Giuseppe Cazzamali, academic research technician Irina Pozdnyakova, postdocs Jesper Langholm Jensen, Magdalena Wiesniewska & Andrea Lages Lino Vala, PhD students Ganesha Pandian Pitchai & Anne Sofie Wanscher, technicians Mia Funk Nielsen, Tasja Ebersole, Christina Lenhard, Alison Lilley & Michael Ross Williamson and laboratory assistant Mille Egeberg Ottosen. Refer to the 'Staff section for a full list of group members.

#### **Internal collaborators**

**Niels Mailand** (Disease Mechanisms Program) on novel SUMO-binding motifs.

**Jiri Lukas** (Disease Mechanisms Program) on the mechanism of Poly(ADP-ribose)-mediated protein accumulation at damaged chromosomes.

Jesper Velgaard Olsen (Proteomics Program) on domain and peptide interaction screens.

**Jakob Nilsson** (Disease Mechanisms Program) on protein-protein interaction studies.

### **Protein Imaging Facility** Associate Professor Claudia Lukas

Contemporary life sciences face an increasing demand on advanced light microscopy to visualize protein localization, interactions, activities and dynamics in their physiological setting, the human cell. Key requirements for cutting-edge light microscopy include high-content applications, interactive live cell imaging, high degree of automation, custom image analysis, and big data management.

To meet these demands and advance the CPR technology pipeline, a new Protein Imaging Facility was established in 2013, which currently houses seven state-of-the-art research microscopes for a broad range of imaging applications. A superbly trained microscopy technician, Jutta Bulkescher, was recruited from the EMBL Heidelberg, Germany to provide highly qualified user support and to maintain microscope instrumentation. In the future, the team is expected to expand by additional staff to implement advanced image analysis and gene editing to tag endogenous proteins by fluorescent markers.

Apart from providing excellent support in microscopy, this activity is a founding example of direct interactions within the Novo Nordisk Foundation Center Cluster, as the new facility is shared and financed on an equal basis between the CPR and DanStem Centers. Despite its short existence, this new cross-center interaction model has proven highly successful in generating an inspiring work atmosphere, allowing access to additional instruments, sharing knowledge, and promoting collaborations.

Other important activities of the new facility include the organization of seminars, workshops, PhD and undergraduate courses, and lively networking with the CFIM microscope Facility at the Faculty of Health and Medical Sciences.

At the CPR side, the facility is coordinated by Associate Professor Claudia Lukas.



### **Disease Systems Biology Program**

The Disease Systems Biology Program uses and develops computational techniques for the joint analysis of molecular, clinical and literature data. The program is led by Professor Søren Brunak and consists of two groups: Cellular Network Biology (Lars Juhl Jensen) and Translational Disease Systems Biology (Søren Brunak).

We have a strong focus on the role of proteins in two major areas, (1) molecular level network biology of mechanisms which operate at the cellular, organ or organismal levels, and (2) integration of clinical data from individuals which can point at proteins which may: become drugs or drug targets, explain disease comorbidities via pleiotropic effects, or rationalize adverse drug reactions by taking human proteome variation into account. Within network biology, the eukaryotic cell cycle is a major theme of the Program, as is systems level analysis of data generated at CPR, for example PTM data. The Disease Systems Biology Program also produces computational tools and resources, which are offered to the research community online.



### **Cellular Network Biology/Jensen Group** Group Leader: Professor Lars Juhl Jensen

The Cellular Network Biology Group was established in January 2009 and is headed by Professor Lars Juhl Jensen. The group is purely computational, using data- and text-mining techniques to understand protein networks. This includes answering biological questions through reanalysis of existing data, developing and maintaining international community resources, and collaborating closely with wet-lab groups both inside and outside of CPR.

Over the past year, the first PhD students and postdocs have finished and successfully transitioned to new positions outside CPR. In 2013, we underwent a five-year evaluation by an international expert panel as the first group at CPR. The evaluation was completed in a highly successful manner with a very positive outcome. After this transition process, the group is now ready to expand from its current composition of one professor and three PhD students.



#### **Research aim**

The overarching theme of the Jensen group is the study of cellular networks and regulation through large-scale data and text mining. The activities in the group fall into three broad categories:

• Network-based analysis of cellular regulation to understand cellular regulation through the analysis of biomolecular networks.

• Development of web-based community resources. Systems analysis of cellular networks requires data to be collected, mined, and unified from heterogeneous sources. The group co-develops a number of webbased community resources.

• Analysis of in-house proteomics data. A major boon of working at CPR is that we are situated next to three proteomics groups, which all produce vast amounts of proteomics data on cellular signaling. This has resulted in many fruitful in-house collaborations.

#### **Key achievements**

The major goal of 2013 was to finalize a suite of web resources that integrate data and text mining to link proteins to the *compartments* where they localize, *tissues* in which they are expressed, and *diseases* in which they are involved. Manuscripts on these have been submitted or are in preparation.

In addition to finalizing the resources described above, we have together with collaborators already used the DISEASES resource to develop a method for linking microRNAs to diseases through their predicted protein-coding target genes (60), and applied our highly efficient text-mining technology to the recognition of taxonomic names in biomedical literature (64).

We have also contributed to new versions of the eggNOG database on the evolution of protein-coding genes (68) and the STITCH database of protein-small molecule associations (46).

#### Internal collaborators

Michael Lund Nielsen (Proteomics Program) on analysis of arginine methylation data. Jesper Velgaard Olsen (Proteomics Program) on analysis of kinase-substrate relationships.

**Søren Brunak** (Disease Systems Biology Program) on analysis of registry data and text mining of electronic health records.

#### Impact and outreach

Our web resources are available to the research community at <u>www.cpr.ku.dk/research/dsb/jensen</u> (direct links <u>http://compartments.jensenlab.org</u>; <u>http://tissues.jensenlab.org</u>; <u>http://diseases.jensenlab.org</u>).

In 2013, the Jensen group has continued its many highly productive collaborations within CPR and the University of Copenhagen. Specifically, we have collaborated with the Proteomics Program on proteomic analyses of PTM-mediated cellular signaling related to drug resistance in breast cancers (*31*) and cardiac targets in beta-adrenergic receptor signaling (*51*).

We have also collaborated with the Proteomics Program and Center for Geogenetics on genomic and proteomic analysis of an ancient horse bone (63).

Finally, we have continued to collaborate with the Translational Disease Systems Biology/Brunak group on text mining of clinical narrative (23) and data mining of electronic health registries (12). In collaboration with the groups of Andrey Rzhetsky and Søren Brunak, we have made an important step forward towards linking analysis of medical data to the molecular level. The major challenge is that we generally do not have access to genetics data on the individuals for which we have medical data. To address this challenge, we analyzed correlations between simple Mendelian disorders and complex genetic diseases (12).

#### **External collaborators**

**Peer Bork** (Bioinformatics, EMBL, Germany) on the STRING, STITCH and eggNOG databases.

**Sean I. O'Donoghue** (Bioinformatics, CSIRO, Australia) on 'COMPARTMENTS' and Reflect resources.

**Andrey Rzhetsky** (Department of Human Genetics, University of Chicago, US) on analysis of medical registry data.

**Evangelos Pafilis** (Institute of Marine Biology HCMR, Greece) on text mining for biodiversity informatics.

**Christian von Mering** (Institute of Molecular Life Sciences, University of Zurich, Switzerland) on the STRING, STITCH and eggNOG databases.

Jan Gorodkin (Department of Veterinary Clinical and Animal Sciences, UCPH, DK) on ncRNA-protein networks.

**Rune Linding** Center for Biological Sequence Analysis, Technical University of Denmark, DK) on deconvolution of signalling networks.

**Eske Willerslev** (Natural History Museum of Denmark , UCPH, DK) on paleoproteomics.

### Translational Disease Systems Biology/Brunak Group Group Leader: Professor Søren Brunak

The group is led by Professor Søren Brunak and consists of 1 guest professor, 2 associate professors, 2 postdocs, 4 PhD students and 1 research assistant. We use and construct computational biology methodology for the analysis of a wide range of data related to proteins in biology and medicine. We have a specific emphasis on broadening an already cross-disciplinary field to include what traditionally has been the domain of 'medical informatics'. Clinical and phenotypic data from the healthcare sector is combined with molecular level data, predominantly genome and proteome information. To improve our understanding of the network biology of individuals, in particular rewired protein interaction networks, we use data on comorbidities obtained from electronic patient records and registries. The aim is to integrate this data in the context of patient stratification, group patients according to their side-effect profiles and further link these to the variation in protein drug targets and off-target effects involving proteins. Another focus is to characterize disease trajectories based on temporal analysis of disease terminology data from patient records across millions of patients. One of the future goals is to use these methods to define cohorts, which may be subjected to screening by clinical proteomics approaches.

#### **Research aim**

The main aims in 2013 have been to complete projects that relate to temporal analysis of patient data and to provide a basic framework for disease trajectory construction from Danish healthcare sector data. Within this overall goal, we have completed two projects that address different time scales in trajectory analysis: Adverse drug reactions (ADRs) occurring during the prescription period of drugs, and lifelong disease terminology trajectories in principle covering all age groups. In terms of data, the two projects were based on electronic patient records and the Danish National Patient Registry (covering the ICD10 era, approximately 15 years), respectively.

#### **Key achievements**

The first project on temporal disease progression analysis aimed at discriminating between treatment related disease-disease correlations and those which may solely have a non-treatment related, genetic origin. Our text mining workflow covers the 7,500 drugs approved for human use in Denmark. We identified multiple statistically significant ADRs and found them to occur at similar frequencies as stated by the manufacturer and in the literature. We showed systematically that drugs displaying similar ADR profiles share protein targets. We also made a dosage-specific analysis and showed a positive correlation between dosage and ADR level for specific drugs. The method identified the potentially fatal adverse event QT prolongation caused by methadone and a non-described likely ADR between levomepromazine and nightmares found among the hundreds of identified novel links between drugs and adverse events.

In the second projec,t we focused on a key prerequisite for precision medicine, namely to estimate future disease progression from the history and current state of a patient. We made a novel, big data analysis of temporal disease development covering 6.2 million Danes. We converted 6.2 million individual trajectories into 1,171 statistically significant directional disease trajectories obtained from data collected over 14.9 years. These trajectories can be grouped into patterns that centre on a smaller number of key diagnoses, such as cardiovascular diseases, Chronic Obstructive Pulmonary Disease (COPD) and gout, which are central to disease progression and hence important to diagnose early to prevent adverse outcomes.

#### Impact and outreach

In an era confronted with data from whole genome sequencing of populations and the emerging clinical proteomics efforts, an ultra-cross disciplinary approach is needed, in particular if the activities should have translational ramifications. We aim for impact in societal terms by training a new generation of researchers to bridge the areas of bioinformatics, systems biology and medical informatics, and by providing new patient stratification methods that may fit the needs of tomorrow's precision medicine. Scientifically, the aim is to make discoveries with multi-functional proteins and their impact on disease comorbidities and temporal disease development.

Our ADR workflow, which is based on text mining technology developed in collaboration with Lars Juhl Jensen, is disease-unspecific and applies to all drugs used in Denmark. It is therefore completely general and of common interest and has already attracted a lot of attention.

#### **Internal collaborators**

Jesper Velgaard Olsen (Proteomics Program) on interaction proteomics.

**Lars Juhl Jensen** (Disease Systems Biology Program) on analysis of registry data and text mining of electronic health records.

#### **External collaborators**

Alfonso Valencia (CNIO, Madrid, Spain) on comorbidities and protein interaction networks.
Pierre Baldi (UC Irvine, California, US) on new machine learning methods for use within medicine.
Andrew Rzhetsky (University of Chicago, US) on meta-analysis of registry and patient record data.
Peter Rossing (Steno Diabetes Center, Gentofte, DK) on data mining of diabetes patient records.
Thomas Werge (Mental Centre Sct. Hans, Roskilde, DK) on data mining of mental disorder patient records.
Anders Juul (Rigshospitalet, Copenhagen, DK) on data mining of growth and reproduction patient records.
Kasper Lage (Broad Institute/Harvard, Boston, US) on disease gene finding and integration of human proteome variation data.

The European ELIXIR Bioinformatics infrastructure consortium Bioinformatics tools registry infrastructure.

**Brunak Group**: Group leader Søren Brunak, associate professor Thomas Blicher, postdoc Anders Boeck Jensen, PhD students Christian Simon & Kalliopi Tsafou, PhD Robert Eriksson and research assistants Andreas Bok Andersen & Isa Kirk. Kalliopi is shared between the Jensen and Brunak groups. Refer to the 'Staff' section for a full list of group members.



### **Proteomics Program**

The Proteomics Program consists of three research groups that develop and apply mass spectrometry-based proteomics to study regulatory post-translational modifications (PTMs) in cell signaling pathways involved in the DNA damage response, receptor signaling as well as metabolic signaling networks.

The program is led by Professor Matthias Mann and comprises three groups headed by Jesper Velgaard Olsen, Michael Lund Nielsen and Chunaram Choudhary. Our broad expertise in PTM analysis covers lysine acetylation and ubiquitin signaling (Chunaram Choudhary), novel approaches for characterizing proteins modified by poly(ADP-ribos)ylation (Michael Lund Nielsen), and *in vivo* phosphoproteomics analyses (Jesper Velgaard Olsen).

We have developed and implemented a world-leading proteomics platform at CPR, which is the technology foundation that gives us exceptional competitive advantage. The Proteomics Program significantly contributes to the CPR protein technology pipeline through its world-leading expertise in high-resolution mass spectrometry and quantitative proteomics screens to identify new proteins and PTMs in cellular model systems and tissue samples. The advanced quantitative proteomics screens performed by the program in collaboration with the other groups at CPR have been the basis of many of the Center's successes so far.

### Mass spectrometry for Quantitative Proteomics/Olsen Group Group Leader: Professor Jesper Velgaard Olsen

The Mass Spectrometry for Quantitative Proteomics Group is headed by Professor Jesper Velgaard Olsen and was one of the first groups to join CPR in 2009. The group currently consists of 4 postdocs, 5 PhD students, 1 research assistant and 1 laboratory technician.

The major scientific focus area for the Olsen group is quantitative, high-resolution mass spectrometry-based proteomics with an emphasis on mass spectrometric technology developments and biological applications. The group has a long-standing interest in applying the proteomics technology developed to systems-wide analyses of dynamic post-translational modifications, e.g. phosphorylation, ubiquitination, acetylation and glycosylation that regulate cell signal transduction pathways. In particular, we are continuously developing the phospho proteomics technology with the aim to be more robust, reproducible, and rapid. The group is also actively developing and applying large-scale quantitative interaction proteomics screens using high-resolution mass spectrometry as the read-out.

#### **Research aim**

The focus of the Olsen group in 2013 included 1) technology development of high-resolution mass spectrometry and tandem mass spectrometry for proteomics, 2) development of 'Quantitative Interaction Proteomics' in tissue samples and 3) quantitative analysis of phosphorylation sites to delineate signaling networks and identify cellular control points.

#### **Key achievements**

In 2013, the Olsen group published 14 peer-reviewed research papers, of which four papers were leading author papers (27, 31, 51) and ten were collaborative (16, 17, 28, 63, 69, 72, 76, 81, 83, 93). This includes publications in top journals such as Nature, Molecular Cell, Nature Genetics and Science Signaling. We have focused on developing and applying phosphoproteomics to



delineate signaling pathways and gained novel insights into the cellular responses. These studies include a system-wide analysis of how TIMP-1 over-expression leads to chemotherapy resistance in breast cancer cells (*31*), and most prominently, we have solved one of the paradigms in FGF signaling by identifying the molecular switch underlying FGF receptor recycling and cell migration (*27*).

To extend the impact of proteomics in biological sciences, we developed robust and reproducible methods to analyze PTMs such as phosphorylation and acetylation, on proteins extracted from tissue samples. This is an important step as it opens new avenues for investigating cell signaling cascades in vivo. In 2013, we for the first time investigated cardiac  $\beta$ -adrenergic signaling on a global scale by analyzing the phosphorylation site changes of proteins extracted from murine hearts treated with  $\beta$ -blockers and -activators (*51, 52*).

#### Impact and outreach

The Olsen group's research attracted some attention in 2013. A publication in Science Signaling about *in vivo* cardiac targets of  $\beta$ -adrenergic receptor signaling (*51*) was highlighted by Faculty of 1000 and led to several popular news stories in Danish media, including <u>videnskab.dk</u> and BT.

We received similar publicity for our publication in Molecular Cell in which we identify the molecular switch underlying FGF receptor trafficking and cellular outputs. This story was the editor's choice in Science Signaling and was highlighted by Danish news media.

The Olsen group continued to attract external funding in 2013, e.g. the 'Genomics History of Denmark' Grant and Manufacturer Vilhelm Pedersen & Wife's Memorial Fund Award for Jesper Velgaard Olsen, and postdoc Alicia Lundby received UNESCO-L'OREAL's For Women in Science Award.

#### **External collaborators**

**Kasper Lage** (Broad Institute MIT and Harvard Medical School, US) on cardiac interaction proteomics. **Eske Willerslev** ((Natural History Museum of Denmark, UCPH, DK) on paleoproteomics/fossilomics. **Alfred Vertegaal** (Molecular Cell Biology, Leiden University Medical Center, the Netherlands) on SUMOyla-

tion in the cell-cycle and DNA damage response. **Blagoy Blagoev** (Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, DK) on growth factor signaling.

**Prasad Jallepalli** (Molecular Biology Program, Memorial Sloan-Kettering, US) on kinase-substrate target identification of cell-cycle regulated kinases

#### **Internal collaborators**

Lars Juhl Jensen (Disease Systems Biology Program) on analysis of kinase-substrate relationships.

Søren Brunak (Disease Systems Biology Program) on interaction proteomics.

Jiri Lukas (Disease Mechanisms Program) on ATR signaling.

Jiri Lukas and Jeremy A. Daniel (Disease Mechanisms Program) on ATM signaling.

Jakob Nilsson and Niels Mailand (Disease Mechanisms Program) on SUMO signaling.

Michael Lund Nielsen and Chunaram Choudhary (Proteomics Program) on proteomics technology.

Mats Wikström (Protein Science Facility) on domain and peptide interaction screens.

Amilcar Flores-Morales (Disease Mechanisms Program) on cancer signaling.

**Olsen Group:** Group leader Jesper Velgaard Olsen, postdocs Chiara Francavilla, Christian Kelstrup & Louise von Stechow, PhD students Jón Sigurdsson, Kristina Emdal, Tanveer Singh Batth, Rosa Jersie-Christensen & Jan Refsgaard Nielsen (the latter is shared between the Olsen and Jensen groups), research assistant Moreno Papetti and technician Dorte Bekker-Jensen (Dorte is shared between the three proteomics groups). Refer to the 'Staff' section for a full list of group members.

### Proteomics Technology Development & Application/Nielsen Group Group Leader: Associate Professor Michael Lund Nielsen

The Proteomics Technology Development and Application Group is led by Associate Professor Michael Lund Nielsen and was established in the autumn of 2009. The group consists of 4 postdocs and 1 PhD student.

The research focus of the Nielsen group is developing new proteomic technologies and implementing these in relevant biological areas. While the goals of proteomics are long-standing, the technology to address them is very challenging and is still in development. This particularly with regard to post-translational modifications (PTMs), which are often present in substoichiometric amounts within the cell compared to their non-modified counterparts. It is therefore advantageous to develop technologies that allow for an up-front enrichment of modified peptides prior to MS analysis (79).

Similarly, interaction proteomics has emerged as a powerful tool to decipher cellular signaling events; however, methodologies for comprehensive characterization of cellular events are currently restricted to certain biological areas. Thus, the current focus area of the group is to build on the analytical capabilities that the proteomics platform provides, and develop novel proteomic technologies and applying these in relevant biological context.

#### **Research aim**

The aim of the Nielsen group is to keep the CPR proteomics infrastructure in a leading position and develop novel proteomic technologies that can be of biological importance for the Center and the research community.

#### **Key achievements**

During 2013, the Nielsen group has (co-)authored 11 publications, of which 4 are published in top tier journals such as Nature, Molecular Cell and Cell Reports (5, 10, 18, 38, 67, 79, 80, 85 and three in press: Christophorou et al: 'Citrullination regulates pluripotency and histone H1 binding to chromatin' Nature; Jungmichel et al.: 'Specificity and commonality of the phosphoinositide-binding proteome analyzed by quantitative mass spectrometry', Cell Reports; Sylvestersen et al.: 'Proteomic analysis of arginine methylation sites in human cells reveals dynamic regulation during transcriptional arrest', Mol Cell Proteomics). Among the proteomic technologies developed by the Nielsen group in 2013 are approaches for characterizing proteins modified by poly(ADP-ribos)ylation (38) and arginine mono-methylation (Sylvestersen et al., in press), and unbiased methods for mapping proteins that directly bind to phosphoinositides (Jungmichel et al., in press).

#### **Internal collaborators**

**Jiri Lukas** (Disease Mechanisms Program) on Poly(ADP-ribos)ylation in the DNA damage response.

Jakob Nilsson & Niels Mailand (Disease Mechanisms Program) on BIO-ID interaction mapping. Jesper Velgaard Olsen (Proteomics Program) on proteomics technology development.

Lars Juhl Jensen (Disease Systems Biology Program) on Bioinformatic analysis of PTMs.

**Amilcar Flores-Morales** (Disease Mechanisms Program) on interaction proteomics of SOCS box family members.

**Jeremy A. Daniel** (Disease Mechanisms Program) on interaction proteomics in the DNA damage response.

The group has collaborated with leading international experts to characterize 'less-well-characterized' modifications such as glutamine methylation and arginine citrullination, and conducted phosphorylation analysis and interaction mapping related to the NBS1-TCOF1/ Treacle complex and the histone chaperone Asf1, respectively.

#### Impact and outreach

The Nielsen group contributes to the research community with the development of novel proteomic technologies that addresses issues of biological importance, as exemplified by the research paper "Proteome-wide Identification of Poly(ADP-Ribosyl)ation Targets in Different Genotoxic Stress Responses" (38), which was awarded the issue cover of Molecular Cell.

#### **External collaborators**

**Tony Kouzarides** (The Gurdon Institute, Cambridge, UK) on glutamine methylation and citrullination. **Manuel Stücki** (IVBMB, University of Zürich, Switzerland) on NBS1-Treacle complex in response to DNA damage.

**Michael O. Hottiger** (IVBMB, University of Zürich, Switzerland) on global characterization of poly(ADP-ribos)ylation targets.

**Philip M. Iannaccone** (Ann & Robert H. Lurie Children's Hospital of Chicago Research Center, Chicago, US) on interaction proteomics of GLI1.

**Norbert Hübner** (Max-Delbrück-Center for Molecular Medicine, Berlin, Germany) on proteogenomics of inbred rats.

**Michal Pravenec** (Institute of Physiology ASCR, Czech Republic) on proteogenomics of inbred rats. **Bernhard Lüscher** (Institute of Biochemistry and Molecular Biology, Aachen University, Germany) on mono-ADP-ribosylation of ARTD10.

**Albert Heck** (Biomolecular Mass Spectrometry and Proteomics, Utrecht University, The Netherlands) on proteomics technology development.

**Anja Groth** (BRIC, UCPH) on interaction profiling of Tousled-like kinases.

**Nielsen Group:** Group leader Michael Lund Nielsen, postdocs Clifford Young, Kathrine Beck Sylvestersen, Rita Martello & Stephanie Jungmichel, research assistant Sara Charlotte Larsen, technician Dorte Bekker-Jensen (Dorte is shared between the three Proteomics groups) and laboratory assistant Maria Vistrup Madsen. Refer to the 'Staff' section for a full list of group members.

### **Proteomics & Cell Signaling/Choudhary Group** Group Leader: Professor Chunaram Choudhary

The Proteomics and Cell Signaling group is headed by Chunaram Choudhary and was established at CPR in the autumn of 2009. The Choudhary group consists of 5 postdocs and 2 PhD students.

In 2013, the group experienced its first major rotation of researchers, one PhD student and three postdocs left the group. Two of the postdocs went on to start their own independent research groups in Germany - Sebastian Wagner started his group at the University of Frankfurt and Petra Beli started her group at the Institute of Molecular Biology (IMB) in Mainz. These departures were offset by the arrival of two new postdocs and two PhD students.



#### **Research aim**

The Choudhary group is interested in investigating the regulatory scope and properties of lysine acetylation, succinylation and ubiquitylation. We have developed proteomics methods for a systems-wide analysis of these PTMs and have provided a detailed view of these modifications in evolutionarily diverse organisms.

#### **Key achievements**

In 2013, the Choudhary group applied a mass spectrometry-based proteomics method for global analysis of succinylation and provided a proteome-wide snapshot of this PTM in evolutionarily diverse organisms (*87*). We showed that succinylation primarily occurs inside the mitochondria, but it is also present in non-mitochondrial compartments. Most acetylation sites in mitochondria overlap with sites modified by acetylation, indicating that many of lysines can be modified by either of these PTMs.

In addition, we investigated the fundamental properties of acetylation and uncovered a new mechanism of lysine acetylation in bacteria (88). The group demonstrated that most acetylation in bacteria depends on the generation of acetyl-phosphate through glyco lysis. Our work showed that acyl metabolites, such as acetyl-CoA and succinyl-CoA, can modify proteins *in vitro*, which suggests that acylation can occur in cells through a non-enzymatic mechanism. These findings have important implications in understanding the functional roles of acetylation and succinylation, particularly in context of their roles in regulating mitochondrial metabolism.

The first author of both of these papers, Brian Weinert, is a postdoc in the group and was the main driving force behind this work.

#### Impact and outreach

The work of the Choudhary group in 2013 has received broad recognition and was covered in the news and views in Molecular Cell and highlighted in the Nature Reviews Microbiology. Group leader Chunaram Choudhary was honored by being selected for the EMBO Young Investigator program.

In 2013, Chunaram Choudhary was invited speaker at five international conferences and an online webinar: 12th HUPO World Congress (Human Proteome Organization annual conference), Yokohama, Japan; FRIAS LifeNet Discussion Meeting on "Proteostasis", Freiburg, Germany; FASEB Summer Research Conference "HDACs, Sirtuins, and Reversible Acetylation in Signaling and Disease," Lucca, Italy; Science Webinar series "Proteomics Meets Cellular Signaling: Exploring Post-translational Modifications by Mass Spectrometry", Online at <u>http://webinar.sciencemag.org/</u>; 5th Summer School in Computational Proteomics," Munich, Germany; Systems Biology of Stem Cells, Kirschberg, Austria

Postdoc Petra Beli was selected for oral presentation at the Keystone meeting "Genomic Instability and DNA Repair (X6)", Banff, Alberta, Canada; and postdoc Brian Weinert was selected for an oral presentation at the 12th HUPO World Congress (Human Proteome Organization annual conference), Yokohama.

#### **External collaborators**

**Ian Hickson** (Center for Healthy Aging, Faculty of Health and Medical Sciences, UCPH, DK) on replication stress signaling.

**Thomas Nystrom** (University of Gothenburg, Sweden) on mechanisms of acetylation in bacteria. **Rudolf Zechner** (University of Graz, Austria) on metabolic regulation of acetylation.

Eric Verdin (University of California, US) on functional investigation of acetylation.

#### **Internal collaborators**

**Niels Mailand** (Disease Mechanisms Program) on ubiquitylation signaling in DNA damage response. **Jeremy A. Daniel (**Disease Mechanisms Program) on analysis of lysine PTMs and protein-protein interaction.

**Choudhary Group:** Group leader Chunaram Choudhary, postdocs Brian Weinert, Christian Schölz, Rajat Gupta, Thomas Wild & Wai Kit Chu, and PhD students Vytautas lesmantavicius & Shankha Satpathy. Refer to the 'Staff' section for a full list of group members.





### **Center Administration** Head of Administration: Peter Dyrsting

The Administration team focuses on providing an excellent first line service to CPR. The team is organized in sub teams specialized within each their area. Each team is partly or fully backed by departments outside the Center, both at the Faculty of Health and Medical Sciences and at the University.

In 2013, Research Coordinator Nanna Rønbjerg Christoffersen, Information Officer Lotte Skipper, Service Assistant Tracy Davis and IT-supporter Sorin Andersen Daescu were recruited to the team, which is now fully staffed with 10 members.

#### Aims and results of the administrative services

The department's primary aims for 2013 have been to (a) contribute to the strengthening of the CPR identity by restructuring the CPR webpage, (b) to organize a management course for the principal investigators, and (c) to follow up on the workplace assessment conducted by the University of Copenhagen in 2012.

(a) In the autumn of 2013, the remaking of the CPR webpage (www.cpr.ku.dk) commenced and the first important steps were taken. The process of strengthening the research group profiles was initiated, the quality of publication lists was improved, staff lists were organized and search engine optimization became an important agenda.

(b) In November 2013, a successful one-week course, the EMBO Laboratory Management Course "The Art of Leadership", was arranged in Copenhagen for all CPR group leaders.

(c) As a consequence of the University's work place assessment, three action plans were designated. The first one was to strengthen leadership skills at group leader level and the EMBO management course was part of this plan. Secondly, to improve the way we disseminate internal information to the employees at CPR. By the end of 2013, the first pages of the CPR intranet were finalized to be published as a part of the intranet at UCPH (<u>www.kunet.dk</u>). Thirdly, we aimed at organizing seminars with focus on work/life balance. These events are planned to take place in 2014.

**Center Administration:** Peter Dyrsting (head of administration), Sorin Andersen Daescu (IT supporter), Camilla Johansson (HR consultant), Mette Efland (personnel administration assistant), Ivan Jensen (laboratory attendee), Johannes Ali Klint (laboratory support), Lotte Skipper (information officer), Bente Larsen Jensen (purchase coordinator), Nanna Christoffersen (research coordinator) and Tracy Davis (service assistant).



### **Training and Education**

Training and education of scientists comprises the foundation that CPR builds upon. Approximately 60% of the Center's current employees are junior researchers, including postdocs, PhD students, and undergraduate students. Thus, training of junior scientists is an integral part of daily life at the Center, providing the scientific community with the next generation of experts within our fields of excellence. As we have a number of group leaders who started their first group at CPR, it is also a priority to provide group leaders with management training possibilities. For this reason, all the group leaders at CPR attended a successful one-week course in November 2013, the EMBO Laboratory Management Course '*The Art of Leadership*'.

In 2013, 6 PhD students students finalized their education and defended their dissertations and 13 postdocs left CPR. A top research center is based on a continuous flow of young scientists in short-term positions and it is our aim that talented scientists perceive CPR as an excellent career move. In this context, it is interesting to analyze the career path of our employees. 90% of our PhD students and postdocs move directly from CPR into jobs in academia and industry. The figures on the right show the distribution of jobs from 2007 to 2013.

CPR strives to be at the forefront of protein technology development and thereby provides a unique training environment for students and postdocs, with state of the art technology. As an example of this, Associate Professor Claudia Lukas organized a three day inhouse microscopy workshop in 2013 ('Workshop on FRAP Microscopy') with two invited speakers and practical hands-on training for 14 students at CPR. Invited speakers were Professor Adriaan Houtsmuller (Erasmus University, Rotterdam) and Dr. Franziska Klein (Training Application & Support Center, Carl Zeiss Microscopy).

In 2013, CPR consolidated a new type of seminars initiated in 2012, called CPR Research in Progress (CPR-IP seminars). Held on a monthly basis, these seminars have two scientific presentations by PhD students or postdocs, each presentation followed by ample time for questions and discussion with the audience. The chair of each session reserves the first five minutes of questions for junior scientists, a simple initiative that has remarkably boosted the scientific discussion. After the seminars, the participants have lunch together, creating a convenient opportunity to interact across the Center programs.

Early steps were taken in 2013 by postdocs at CPR to organize an interest group, the so-called ASAP (Association of Students and Postdocs), which will meet every two weeks to exchange technological knowledge, discuss career development, plan events, etc. ASAP will join the group leaders in inviting speakers for seminars hosted by CPR and will arrange for speakers to interact with interested postdocs and students in a closed session.

# Employment of former CPR PhD students (9 total)



#### Undergraduate teaching at the Faculty

A number of junior and senior scientists at CPR took part in undergraduate teaching activities in 2013: Postdoc Kathrine Beck Sylvestersen and associate professor Michael Lund Nielsen taught in the 'Advanced protein science I' course for master students. Postdoc Kasper Harpsøe taught in the master level course 'Structural & Computational Medicinal Chemistry'. Associate professor Thomas Blicher, PhD student Anders Boeck Jensen and professor Lars Juhl Jensen, all from the Disease Systems Biology Program, arranged and executed the bioinformatics part of the Human Biology master course 'Molecular Biology, *Bioinformatics & Cell Biology'*. PhD student Rune Busk Damgaard taught in two bachelor level courses at Institute for Cellular and Molecular Medicine (ICMM). PhD student Sabrina Gade Ellesøe taught bachelor level medical students in a practical course. Finally,

associate professor Jeremy A. Daniel held immunology lab exercises for bachelor students of Molecular Biomedicine.

The elective master course 'Advanced Methods for the analysis of protein disease mechanisms' was prepared in 2013 and will take place at CPR in the fall of 2014. The course covers subjects across the Center's research programs and aims to accommodate students from 3 different master programs: Human Biology, Molecular Biomedicine, and Biochemistry (Faculty of Science).

Scientists at CPR wish to contribute more to undergraduate teaching at the Faculty of Health and Medical Sciences. While providing a chance to share knowledge and inspire future scientists, it is also a fruitful chance to recruit future master and PhD students to the Center, as exemplified by the case story below. As part of the comprehensive strategic development carried out at CPR in 2013, the management has taken steps to integrate the Center's expert scientists even more as an active part of the undergraduate training taking place at our Faculty and we look forward to collaborate with the Faculty to integrate these measures in the coming years.

#### **Graduate teaching**

CPR organizes several biennial PhD courses, which will be offered next time in 2014. In addition, CPR scientists contribute to graduate teaching at other research institutions, as expert authorities of their fields.

In 2013, professor Lars Juhl Jensen contributed to graduate courses in Germany, Turkey and Australia (Bioinformatics Spring School, Seeon-Seebruck, Germany, MaxQuant Summer School, Martinsried, Germany, EMBO Practical Course on Computational Biology, Nevsehir, Turkey, and EMBL-Australia Masterclass on Protein Sequence Analysis, Sydney, Australia). Associate professor Michael Lund Nielsen taught in the PhD course 'Biomolecular Mass Spectrometry & Proteomics' in Utrecht, the Netherlands. Postdoc Diego Iglesias-Gato taught in the course 'Survey of Molecular Endocrinology', a theoretical course in the Doctoral Program in Metabolism and Endocrinology at the Karolinska Institute in Stockholm, Sweden. Finally, postdoc Kasper Harpsøe taught in the PhD course 'Biostructures and Molecular Modeling in Drug Research' at the Department of Drug Design and Pharmacology (Faculty of Health and Medical Sciences, UCPH).

#### **Interview: Master student Laura Maarit Pikkupeura**

Laura Maarit Pikkupeura joined the Chromatin Structure & Function Group/Daniel Lab at CPR as a master student in 2013. At that time, she had already decided to move from Uppsala to the University of Copenhagen, to join the Molecular Biomedicine master program.

A lecture that Jeremy A. Daniel gave for her immunology course drew her attention towards his research and CPR.

'I was hoping to work with DNA damage and epigenetics, so his research group seemed like a perfect match for me.'

After this initial introduction to CPR, Laura did some background research on the Center and was attracted by its multidisciplinary profile and the combination of basic and applied research. Now, having worked at CPR for the past 6 months, she has experienced the CPR scientific environment and spirit for herself.

'CPR provides advanced equipment, a wide range of expertise and an opportunity to establish collaborations with researchers from multidisciplinary backgrounds. I enjoy the young, enthusiastic and international atmosphere. Furthermore, I expect to gain methodological proficiency in a wide range of technologies and advanced knowledge in chromatin biology. I also look forward to establishing collaborations vital for my future career', she concludes.



### **Scientific Output**

In 2013, CPR has once again increased its productivity with regard to publishing papers in peer-reviewed journals. More than 90 research papers were accepted in scientific journals, including Nature, Science, Cell, Molecular Cell and Nature Genetics.



Number of papers authored or co-authored by CPR scientists.



CPR papers 2008-2013

CPR papers published in journals with impact factor  $\geq$ 10 (impact factors are given in brackets).

### **CPR Key Facts**

The diagrams represent key data about CPR from 2013.



### **Research Grants and Awards**

2013 proved to be another successfull year for attracting external funding to CPR. In total, more than DKK 28 M was obtained in external funding.

#### Awards & Honors 2013

Awards and honors that were awarded in 2013.

Professor Niels Mailand was awarded an ERC grant and also received the Silver Medal for young Danish researchers awarded by the Royal Danish Academy of Science and Letters. Professor Niels Mailand and Professor Chunaram Choudhary joined the EMBO Young Investigators network. Postdoc Matthias Altmeyer was awarded with the Sapere Aude DFF-Research Talent and finally, postdoc Andreas Mund and postdoc Kai Neelsen were awarded the EU Marie Curie IEF grant.

#### **Danish Public Grants**

Grants that were awarded and/or initiated in 2013.

The Danish Council for Independent Research (Medical Sciences) granted postdoc Godelieve Smeenk DKK 1.1 M ( $\in 0.14$  M) for the project 'Identification of novel regulators of translesion DNA synthesis in human cells' and also granted postdoc Matthias Altmeyer DKK 2.5 M ( $\in 0.33$  M) for the project 'Identification and characterization of concealed regulators of genome maintenance pathways'.

Moreover, Associate Professor Jeremy Daniel received DKK 7.0 M ( $\in$  0.94 M) for the project "Mechanism and specificity of PTIP complex function in B lymphocytes". The grant is donated as the second step in the Sapere Aude research career program: DFF-Starting Grant. The Danish Council for Independent Research (Medical Sciences) also granted postdoc Linda Starnes DKK 3.0 M ( $\in$  0.4 M) for the project '*Elucidating the function of ATM kinase activity during development*'.

#### **Danish Private Grants**

Grants that were awarded and/or initiated in 2013.

The Novo Nordisk Foundation granted DKK 1.3 M ( $\in$  0.17 M) to Associate Professor Claudia Lukas for the project 'Spatial-temporal profiling of genome integrity maintenance', DKK 2.0 M ( $\in$  0.27 M) to Professor Niels Mailand for the project 'Functional characterization of DVC1, a new key protein in the DNA damage response' and DKK 1.0 M ( $\in$  0.13 M) for the project 'Tumor Suppressor functions of SOCS proteins in prostate cancer' to Professor Amilcar Flores-Morales.

From the Lundbeck Foundation, Associate Professor Simon Bekker-Jensen received DKK 1.5 M ( $\in$  0.2 M) for the project 'Structural and functional characterization of a novel class of SUMO-binding domains' and Associate Professor Mads Gyrd-Hansen received DKK 1.0 M (€ 0.13 M) for the project '*Regulation of NOD2-dependent immune signaling by ubiquitin and its impact in the human immunodeficiency syndrome XLP2*'.

The Danish Cancer Society granted Professor Niels Mailand DKK 2.3 M ( $\in$  0.3 M) for the project 'Molecular regulation of translesion DNA synthesis, a double-edged sword in cancer development'. In addition, the Danish Cancer Society granted Professor Jiri Lukas DKK 1.6 M ( $\in$  0.21 M) for a project initiated prior to his arrival at CPR.

#### **International Grants**

Novo Nordisk - Chinese Academy of Sciences Research Foundation granted Associate Professor Mats Wikström DKK 0.4 M (€ 0.05 M) for the project 'Biophysical and Structural Studies of the Interaction Matrix of IGFBPs'.

The Movember Foundation granted Professor Amilcar Flores-Morales DKK 2.7 M ( $\in$  0.36 M) for the project *'Mass spectrometry based proteome profiling for precision medicine in prostate cancer'*.



### Staff

A comprehensive list of all employees and affiliated personnel at CPR during 2013, organized by research program and group. The list also includes employees that left or joined CPR in 2013.

**Disease Mechanism Biology Program** Jiri Lukas Program Director

**Chromosome Stability and Dynamics** Jiri Lukas Group Leader, Professor

Claudia Lukas Associate Professor

Dorthe Helena Larsen Postdoc

Kai Neelsen Postdoc

Luis Toledo Lazaro Postdoc

Matthias Altmeyer Postdoc

Ronni Sølvhøj Pedersen PhD Student

Thorkell Gudjónsson PhD Student

Maj-Britt Druedahl Rask Technician

Merete Grøfte Technician

Andreas Willems Laboratory Assistant

**Chromatin Structure and Function** Jeremy Austin Daniel Group Leader, Associate Professor

Andreas Mund Postdoc

Su Dan Postdoc

Linda Starnes Postdoc Laura Pikkupeura Master Student

Rebeca Soria Romero Technician

Mette Flinck Laboratory Assistant

**Mitotic Mechanisms and Regulation** Jakob Nilsson Group Leader, Associate Professor

Daniel Geoffrey Hayward Postdoc

Thomas Kruse Postdoc

Garry Gray Sedgwick Postdoc

Gang Zhang Postdoc

Tiziana Lischetti Postdoc

Julie Schou PhD Student

Marie Sofie YooLarsen PhD Student

Jamin Hein PhD Student

Stephanié Holstein-Rathlou Laboratory Assistant

Ida Marie Boisen Laboratory Assistant

Molecular Endocrinology Amilcar Flores-Morales

Group Leader, Professor Diego Iglesias-Gato

Postdoc

Indranil Paul Postdoc Kim Hjorth-Jensen Postdoc

Charlotte Svensson PhD Student

Anne-Sophie Fenger Bachelor Student

Matilde Hyldig Laboratory Assistant

**Ubiquitin Signaling** Niels Mailand Group Leader, Professor

Tina Thorslund Associate Professor

Mads Gyrd-Hansen Associate Professor

Simon Bekker-Jensen Associate Professor

Berthe Fiil Postdoc

Lou Klitgaard Povlsen Postdoc

Sara Lund Poulsen Postdoc

Godelieve Smeenk Postdoc

Petra Schwertman Postdoc

lan Gibbs-Seymour Postdoc

Yasuyoshi Oka Postdoc

Anita Ripplinger PhD Student

Anna Mosbech PhD Student

Bine Hare Villumsen PhD Student Maria Poulsen PhD Student

Maxim Tollenaere PhD Student

Rebecca Kring Hansen PhD Student

Rune Busk Damgaard PhD Student

Saskia Hoffmann PhD Student

Stine Smedegaard PhD Student

Louise Aagaard Nilausen Master Student

Rikke Høgh Bachelor Student

Christina Råe Hansen Laboratory Assistant

**Protein Science Facility** Mats Wikström Group Leader, Associate Professor

Werner Streicher Academic Coordinator

Giuseppe Cazzamali Academic Coordinator

Tobias Gustavsson Academic Coordinator

Tine Kragh Nielsen Academic Coordinator

Irina Pozdnyakova Academic Research Technician

Diana Huttner Postdoc

Jesper Langholm Jensen Postdoc

Magdalena Wiesniewska Postdoc

Andrea Lages Lino Vala Postdoc Stefan Kol Postdoc

Carl Diehl Postdoc

Ganesha Pandian Pitchai PhD Student

Anne Sofie Wanscher PhD Student

Sofia Møller Master Student

Diana Skousborg Larsen Technician

Havva Koc Technician

Mia Funk Nielsen Technician

Tasja Ebersole Technician

Khalid Pardes Technician

Christina Lenhard Technician

Marina Jeppsson Technician

Michael Ross Williamson Technician

Mille Egeberg Ottosen Laboratory Assistant

**Protein Imaging Facility** Jutta Maria Bulkescher Technical Assistant

**Disease Systems Biology Program** Søren Brunak Program Director, Professor

**Translational Disease Systems Biology** Søren Brunak Group Leader, Professor

Pope Mosely Professor Thomas Blicher Associate Professor

Thomas Frimurer Associate Professor

Anders Boeck Jensen Postdoc

Kasper Harpsøe Postdoc

Peter Bjødstrup Jensen Postdoc

Sabrina Gade Ellesøe PhD Student

Christian Simon PhD Student

Kalliopi Tsafou PhD Student

Michael Lückmann Master Student

Isa Kirk Research Assistant

Andreas Bok Andersen Research Assistant

Piotr Dworzynski IT Programmer

#### Cellular Network Biology

Lars Juhl Jensen Group Leader, Professor

Sune Pletscher-Frankild Assistant Professor

Heiko Horn Postdoc

Jan Refsgaard Nielsen PhD Student

Alberto Santos Delgado PhD Student **Proteomics Program** Matthias Mann Program Director, Professor

Mass Spectrometry for Quantitative Proteomics Jesper Velgaard Olsen, Group Leader, Professor

Alicia Lundby Postdoc

Chiara Francavilla Postdoc

Christian Kelstrup Postdoc

Louise Knudsen Postdoc

Louise von Stechow Postdoc

Omid Hekmat Postdoc

Ida Kjær Christensen PhD Student

Jón Sigurdsson PhD Student

Kristina Bennet Emdal PhD Student

Stephanie Munk PhD Student

Tanveer Singh Batth PhD Student

Rosa Jersie-Christensen PhD Student

Moreno Papetti Research Assistant

Dorte Bekker-Jensen Technician **Proteomics and Cell Signaling** Chunaram Choudhary Group Leader, Professor

Brian Weinert Postdoc

Christian Schölz Postdoc

Petra Beli Postdoc

Rajat Gupta Postdoc

Sebastian Wagner Postdoc

Thomas Wild Postdoc

Wai Kit Chu Postdoc

Peter Henriksen PhD Student

Vytautas lesmantavicius PhD Student

Shankha Satpathy PhD Student

#### **Proteomics Technology Development and Application** Michael Lund Nielsen Group Leader, Associate Professor

Clifford Young Postdoc

Christian Toft Madsen Postdoc

Kathrine Beck Sylvestersen Postdoc

Rita Martello Postdoc

Stephanie Jungmichel Postdoc

Jon Wriedt Poulsen PhD Student Sara Charlotte Larsen Research Assistant

Troels Mygind Poulsen PhD Student

Zacharias Damholt Master Student

Heidi Grell Technician

Maria Vistrup Madsen Laboratory Assistant

**Center administration** Peter Dyrsting Head of Administration

Anatoliy Dmytriyev Head of Systems Administration

Sorin Andersen Daescu IT Support

Gitte Thorvil IT Engineer

Camilla Johansson HR Consultant

Mette Efland Personnel Administration Assistant

Ivan Jensen Laboratory Attendee

Johannes Ali Klint Laboratory Support

Olivier Bitterlin Laboratory Engineer

Lotte Skipper Information Officer

Bente Larsen Jensen Purchase Coordinator

Anni Thomsen Research Coordinator

Nanna Rønbjerg Christoffersen Research Coordinator

Tracy Davis Service Assistant



### **Publications**

The list includes papers published in 2013 *or* published online ahead of print in 2013. Papers mentioned in the annual report as *in press* are defined as 2014-publications and are thus not listed here.

1. Absalom, NL, Quek, G, Lewis, TM, Qudah, T, von Arenstorff, I, Ambrus, JI, Harpsøe, K, Karim, N, Balle, T, McLeod, MD & Chebib, M 2013, 'Covalent Trapping of Methyllycaconitine at the α4-α4 Interface of the α4β2 Nicotinic Acetylcholine Receptor: ANTAGONIST BINDING SITE AND MODE OF RECEPTOR INHIBITION REVEALED' *The Journal of Biological Chemistry*, vol 288, no. 37, pp. 26521-32., http://dx.doi.org/10.1074/jbc.M113.475053

2. Altmeyer, M & Lukas, J 2013, 'Guarding against Collateral Damage during Chromatin Transactions' *Cell*, vol 153, no. 7, pp. 1431-4., http://dx.doi.org/10.1016/j.cell.2013.05.044

3. Altmeyer, M, Toledo Lazaro, LI, Gudjonsson, T, Grøfte, M, Rask, M-B, Lukas, C, Akimov, V, Blagoev, B, Bartek, J & Lukas, J 2013, 'The Chromatin Scaffold Protein SAFB1 Renders Chromatin Permissive for DNA Damage Signaling' *Molecular Cell*, vol 52., http://dx.doi.org/10.1016/j.molcel.2013.08.025

4. Altmeyer, M & Lukas, J 2013, 'To spread or not to spread - chromatin modifications in response to DNA damage' *Current Opinion in Genetics & Development*, vol 23, no. 2, pp. 156-165., http://dx.doi.org/10.1016/j. gde.2012.11.001

5. Andresen, CA, Smedegaard, S, Sylvestersen, K, Svensson, C, Iglesias-Gato, D, Cazzamali, G, Nielsen, TK, Nielsen, ML & Flores-Morales, A 2013 'Protein Interaction screening for the ankyrin repeats and SOCS box (ASB) family identify Asb11 as a novel ER resident ubiquitin ligases' *The Journal of Biological Chemistry*', vol 289, pp. 2043-2054 (Epub 2013/12/11), http://dx.doi: 10.1074/jbc.M113.534602

6. Audouze, KML, Brunak, S & Grandjean, P 2013, 'A computational approach to chemical etiologies of diabetes' *Scientific Reports*, vol 3, 2712, pp. 2712., http://dx.doi.org/10.1038/srep02712

7. Bager, RJ, Persson, G, Nesta, B, Soriani, M, Serino, L, Jeppsson, M, Nielsen TK & Bojesen, AM 2013 'Outer membrane vesicles reflects environmental cues in Gallibacterium anatis' *Veterinary Microbiology*, vol. 167, pp. 565-572, http://dx.doi.org/10.1016/j.vetmic.2013.09.005

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