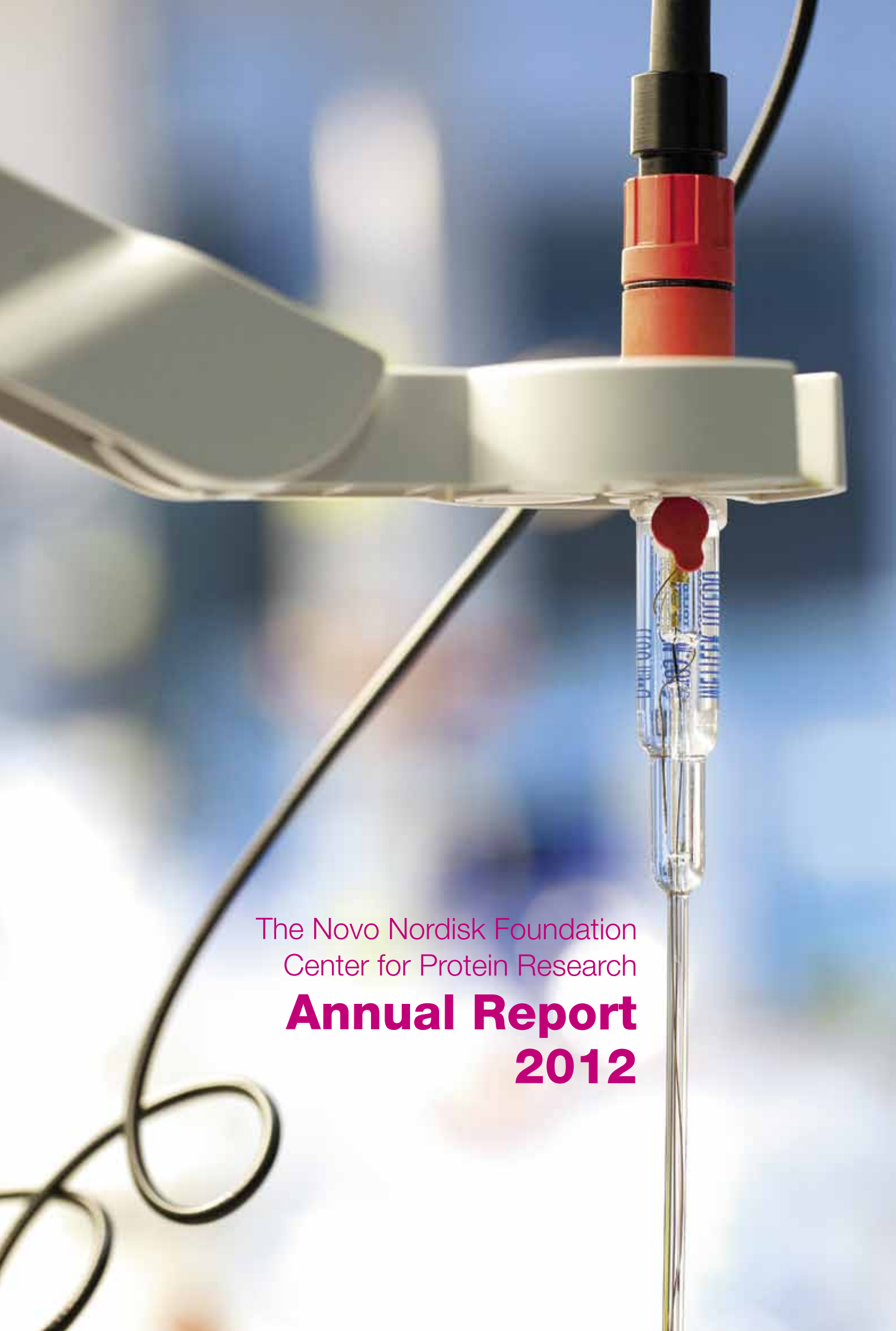


The Novo Nordisk Foundation
Center for Protein Research

Annual Report 2012

Contents

Progress and Performance in 2012

In 2012, the first half of the Novo Nordisk Foundation funding period came to an end. Consequently, the past year was in part characterized by evaluation of our scientific progress in relation to the goals that were originally set out. After five years of existence, we are proud to report that all major milestones have been met and that CPR now comprises a wide range of expertise and skills in protein science, with activities in disease systems biology, proteomics and disease biology. The research departments are supported by a core facility for high-throughput protein production and characterization. The core facility has recently been reconfigured to become much more project-oriented and tailor made to meet the increasing in-house demands associated with the transition to the second funding period, where the research output will be the key criterion for success. CPR has become a research institute with hallmarks of excellence such as its state-of-the-art infrastructure, an organization that strongly promotes internal collaboration, and a very supportive and dynamic atmosphere. We have continued to attract the best talent from around the world and currently accommodate ~140 scientists and support staff with more than 25 different nationalities. Most significantly, the scientific output during the last three years has been very competitive and forms a solid basis for fulfilling the key ambition of CPR to become a world-leading research center in protein biology. In 2012 more than 60 scientific papers were accepted in a number of high-impact journals including Cell, Molecular Cell, Nature Structural & Molecular Biology, Nature Cell Biology and Nature Reviews. The individual groups have made great progress in the development of new and efficient proteomic techniques, and in revealing the role of novel proteins and their function in the cell signaling pathways involved in processes such as DNA repair, mitosis and genome stability.

During 2012 the CPR management underwent important development. Prof. Jiri Lukas was appointed new Executive Director from April 1st, 2012 taking over from the interim manager, Jesper Velgaard Olsen. Jiri Lukas is world-renowned for his seminal discoveries in DNA damage signaling and cancer biology. He was recruited from the Danish Cancer Society, where he was the director of the Danish National Research Foundation Center for Genotoxic Stress Research. At CPR, Jiri Lukas also leads the Chromosome Stability and Dynamics group, the Department of Disease Biology. Jesper Velgaard Olsen will remain closely involved in the CPR management as Vice Director. Additionally, the administration was strengthened by the recruitment of Peter Dyrsting as Head of Administration from April 1st, 2012. Peter Dyrsting holds a Master of Science in Economics and Business Administration from Copenhagen Business School and he previously was Finance and Planning Manager at the Department of Odontology at the Faculty of Health and Medical Sciences, University of Copenhagen.

The integration of the Lukas group, with its expertise in advanced protein imaging and genetic screens, adds a complementary and powerful approach to the CPR analytical portfolio by providing the possibility to identify and functionally dissect protein pathways in a high-throughput fashion.

One of the most important events for CPR in 2012 was the very successful 2nd Copenhagen Bioscience Conference: PTMs in Cell Signaling. The conference was hosted by the Novo Nordisk Foundation and co-organized by CPR. More than 200 renowned protein researchers gathered at the Comwell Borupgaard north of Copenhagen for four intensive days of scientific discussions, landing a new tradition for regular discussion and development of this important field of protein science.

Jiri Lukas
Executive Director

Jesper Velgaard Olsen
Vice Director

Profile of the New Executive Director, Prof. Jiri Lukas

Jiri Lukas is internationally recognized for his seminal discoveries in DNA damage signaling and cancer biology. He was recruited from a position as Director of the Danish National Research Foundation Center for Genotoxic Stress Research at the Danish Cancer Society.

'We deliberately chose a director with a top research profile because the Center is now moving from the start-up phase, with focus on infrastructure and establishment of research environments, to phase two, with full attention on research activities,' says Matthias Mann, one of the CPR founders and research director of the Department of Proteomics. Dean of the Faculty of Health and Medical Sciences, University of Copenhagen, Ulla Wewer adds that 'his appointment is of enormous importance to the progress of the Center'.

Apart from his strong desire to remain an active scientist, Jiri Lukas' ambition is to provide authoritative guidance in terms of strategic research priorities to all group leaders, to actively promote their mutual interactions at all levels, and to harness the potential of the Center to synergize both at the intellectual and technological levels.

'From the perspective of the Executive Director, the most gratifying experience has been to see that during its relatively short period of existence (2-3 years for most groups) the CPR has established itself not only as the hub of state-of-the-art protein technology but also as an institute that has already generated high-profile discoveries in some of the most competitive areas of protein research,' says Jiri Lukas.

Professional Background

Prof. Jiri Lukas studied veterinary medicine and holds a PhD from the Czech Academy of Sciences. As a postdoc he worked with Noble Laureate, Paul Nurse in Oxford and Giulio Draetta at the European Molecular Biology Laboratory (EMBL) in Heidelberg. In 1993 Jiri Lukas moved to the Danish Cancer Society, where, together with Jiri Bartek and Michael Strauss, he created a dynamic laboratory focused on cell cycle regulation and cellular responses to DNA damage. He has received numerous awards for his research, including the Novo Nordisk Prize and the Mendel Medal, and he holds prestigious memberships, e.g. in the European Molecular Biology Organization (EMBO) and the Royal Danish Academy of Sciences and Letters. He has written or contributed to more than 160 publications in major journals and has been shortlisted as one of the most influential scientists in molecular genetics and genomics in the past decade. Jiri Lukas' scientific interests cover the behavior of chromosomes when cells divide and when they experience DNA damage; he focuses on the role these mechanisms play in diseases associated with genetic instability, such as cancer. His work has been instrumental in developing research tools to directly visualize, in living human cells, the ways proteins interact with damaged chromosomes.

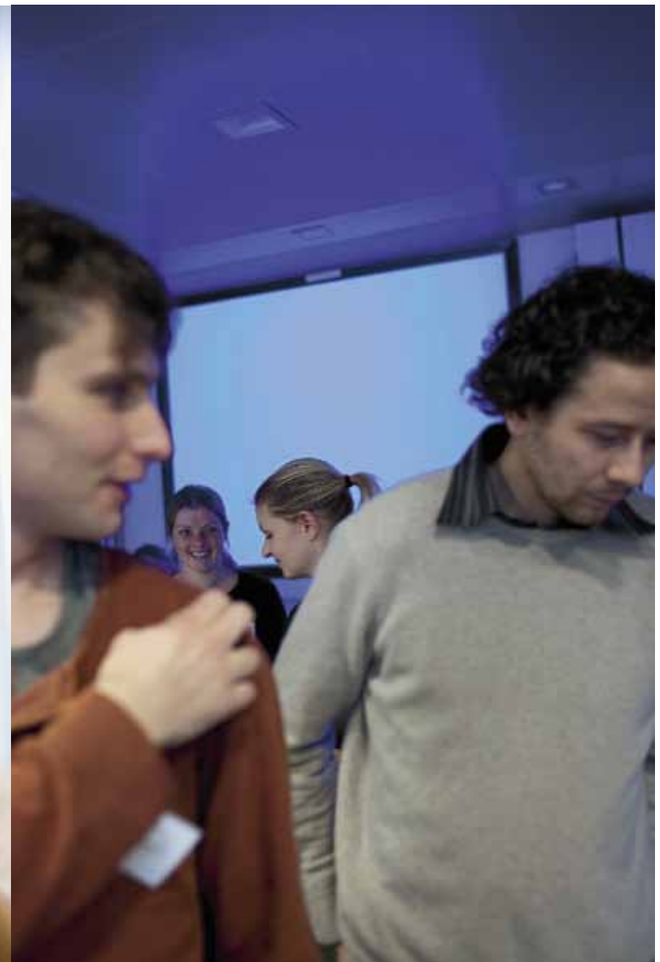
Jiri Lukas

Chairman of the Scientific Advisory Board

As with previous meetings of the Scientific Advisory Board (SAB), the Board was impressed with the scientific achievements of the CPR scientists over the previous year, and the increasing progress in securing competitive grants to complement the Novo Nordisk Foundation funding. The Board was particularly impressed with the vision, actions, and science of the new Executive Director, Prof. Lukas.

Since its founding, CPR has rapidly built an impressive program that combines protein science, proteomics, computational science, and disease biology. In last year's Annual Report, the SAB noted that for CPR to leverage these strengths from its start-up phase to meet its full potential, adjustments would be needed in a number of operational and scientific areas. CPR has made these adjustments, and the Board feels very confident that under the leadership of Prof. Lukas, the Center is in excellent state for the upcoming 5-year review of its programs by the Novo Nordisk Foundation, and is poised to realize its enormous potential for international leadership in protein science and biology

Christopher Austin
Chairman of the SAB 2012
Director of the NIH Chemical Genomics Center
Bethesda, US



Boards

A crucial management asset of any ambitious research institute is a functional Scientific Advisory Board (SAB). We are fortunate to have eminent scientists on the SAB; true authorities and paradigm-makers in their respective fields, whose opinion and advice have been instrumental in guiding the CPR management both on logistic and scientific issues. The SAB and CPR management convened in June 2012. At the meeting the group leaders presented the work of their groups and engaged in dialogue with the members of the SAB.

Scientific Advisory Board

- **Dr. Christopher Austin** (Chair), Director of the National Institutes of Health (NIH) Chemical Genomics Center (NCGC), Bethesda, Maryland (US)
- **Dr. Anthony A. Hyman,** Group Leader and Director at the Max Planck Institute of Molecular Cell Biology and Genetics, Dresden (Germany)
- **Prof. Poul Nissen,** Principal Investigator at the Department of Molecular Biology, Center for Structural Biology, University of Aarhus (Denmark)
- **Prof. Torben Ørntoft,** Head of Department of Molecular Medicine, Aarhus University Hospital at Skejby (Denmark)
- **Prof. Pernille Rørth,** Research Director, Institute of Molecular and Cell Biology, A-STAR (Singapore)
- **Prof. Ivan Dikic,** Director of the Institute of Biochemistry, Goethe University, Frankfurt and Scientific Director of the FMLS/Frankfurt Institute for Molecular Life Sciences (Germany)
- **Prof. Tony Pawson,** Senior Investigator at the Samuel Lunenfeld Research Institute, Toronto (Canada)

In 2012 it was decided to expand the SAB to fully cover the fields that have been added to the CPR portfolio after the recent arrival of new groups. Thus, we would like to welcome two new members of the SAB: Dr. Andre Nussenzweig (NIH) and Prof. Angus Lamond (University of Dundee), who are world-leading experts in genome integrity and proteomics/advanced imaging, respectively. The SAB is designed to rotate frequently and the Chairman, Prof. Chris Austin, stepped down at the end of 2012, transferring the chairmanship to Prof. Tony Hyman. We would like to thank Prof. Chris Austin for his time and valuable contribution to our Scientific Advisory Board.

Steering Committee

The Steering Committee is composed of an independent panel of distinguished members with different professional backgrounds to provide advice and governance regarding financial, management and strategic matters.

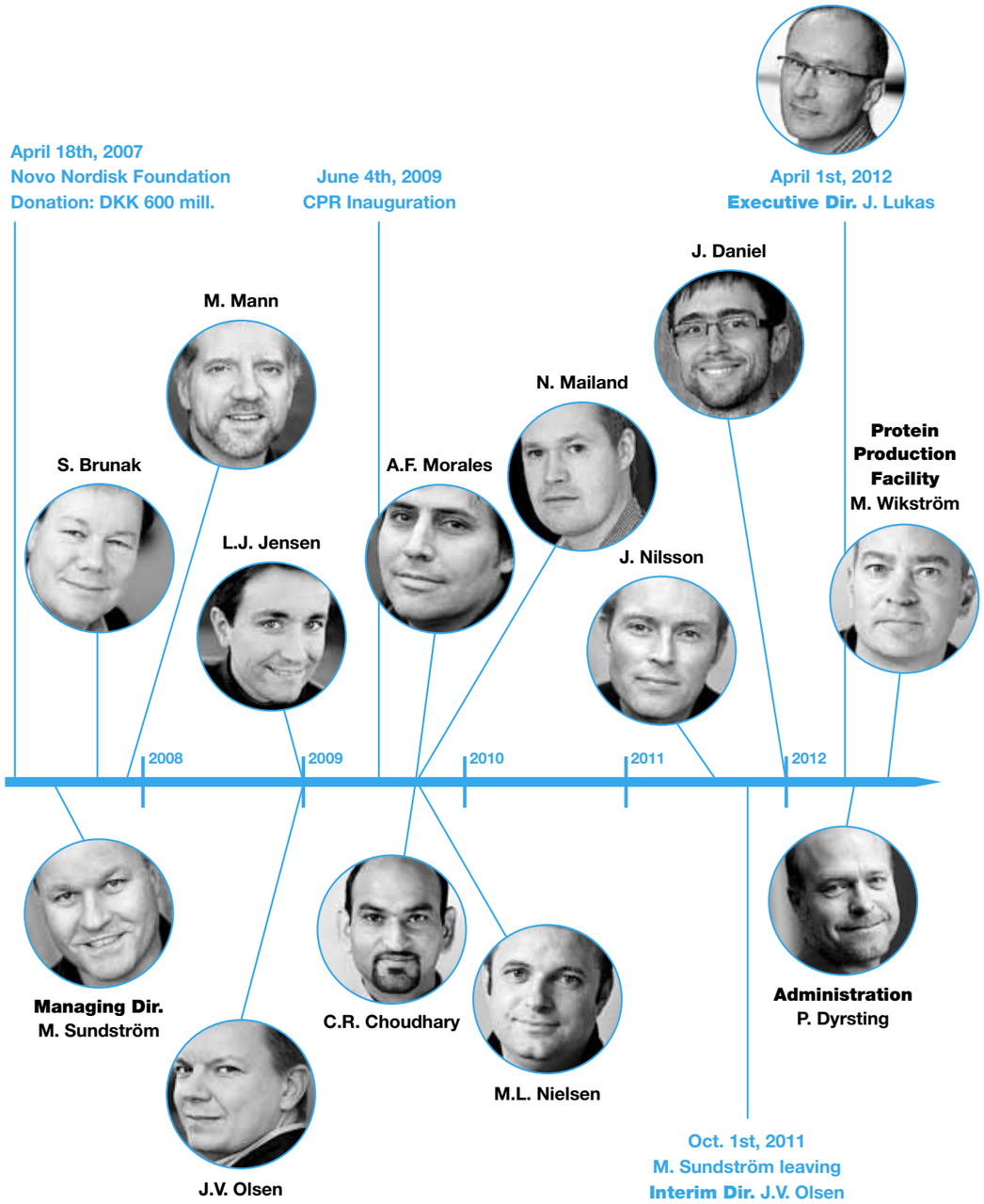
We are grateful for the valuable contributions from the members of the Steering Committee that consists of:

- **Ulla Wewer** (Chair), Dean of the Faculty of Health and Medical Sciences, University of Copenhagen
- **Nils O. Andersen,** Commissioner for Rector, University of Copenhagen
- **Per Holten-Andersen,** President of Copenhagen Business School
- **Henrik Wegener,** Prorector of the Technical University of Denmark
- **Ulla Brockenhuus-Schack,** Managing Director, Seed Capital
- **Ingemar Carlstedt,** Assistant Dean of the Faculty of Medicine, Lund University
- **Leif Beck Fallesen,** Business Policy Adviser, Business Commentator. Municipality of Copenhagen, TV2/ Denmark
- **Sven Frøkjær,** Associate Dean of the Faculty of Health and Medical Sciences, University of Copenhagen
- **Lars Goldschmidt,** Managing Director, Danish Association of Consulting Engineers
- **Jannik Hilsted,** Medical Director, Copenhagen University Hospital
- **Kim Høgh,** Group Managing Director, Capital Region of Denmark
- **Ida Sofie Jensen,** Managing Director, Danish Association of the Pharmaceutical Industry (LIF)
- **Mathias Uhlén,** Prof., Royal Institute of Technology (KTH), Stockholm

Community Target Committee

- **Dr. Peter Andreasen,** Department of Molecular Biology, Aarhus University.
- **Prof. Lars Björck,** Department of Clinical Sciences, Division of Infection Medicine, Lund University, Sweden
- **Prof. Henrik Ditzel,** Center for Medical Biotechnology, Institute of Medical Biology, University of Southern Denmark, Odense
- **Prof. Anders Lund,** Copenhagen BioCenter, Biotech Research and Innovation Centre, University of Copenhagen
- **Dr. Michael Toft Overgaard,** Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Denmark
- **Prof. Ole William Petersen,** Department of Medical Anatomy, University of Copenhagen
- **Prof. Thue Schwartz,** Department of Molecular Pharmacology, Institute for Neuroscience and Pharmacology, University of Copenhagen
- **Prof. Kristian Strømgaard,** Department of Medicinal Chemistry, Faculty of Health and Medical Sciences, University of Copenhagen
- **Prof. Birte Svensson,** Institute for Systems Biology, Enzyme and Protein Chemistry, Technical University of Denmark
- **Dr. Mats Wikström** (Chair), Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen

CPR Timeline



The competence of the people working here, and the core funding, provide the opportunities for highly ambitious long-term scientific endeavors and opportunities for a unique synergy among the departments that in many ways can be regarded as interdisciplinary. With its unique setup CPR has been able to attract some of the most talented young leaders in the areas of proteomics, systems biology and disease biology. Most of them have started their own research groups for the first time and this has worked out perfectly, reflecting positively on the mentoring by the senior research directors. A distinct focus has been, and will continue to be, on allowing for independent group leaders to grow and develop their skills in scientific management and leadership.

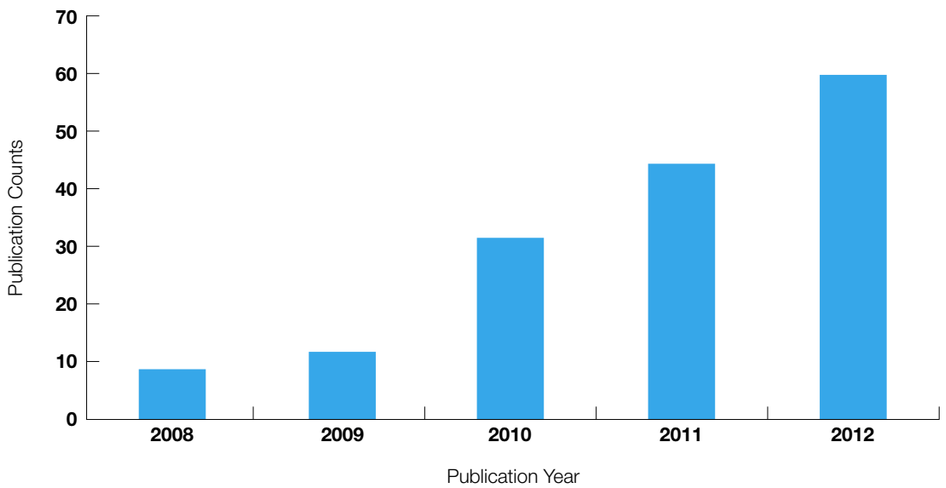
Progress Towards Goals – Midterm Evaluation Reflections

To reach our goal of becoming a world-leading research center in protein research, we have used advanced technology-driven approaches combined with mechanistic studies to analyze specific areas of disease biology and thereby generate fundamental insights into protein function in health and disease. We have created a highly competitive and international work environment with a strong identity, and succeeded in establishing state-of-the-art infrastructure and platforms. We run a large high-performance computing cluster with six of the latest generation of Orbitrap mass spectrometers and several state-of-the-art microscopes including imaging facilities capable of high-content genetic screens using sub-cellular protein dynamics as a readout. Furthermore, we have successfully implemented a protein production platform that permits large-scale expression of native and full-length proteins in *E. coli*, insect cells and human cells. Finally, the computational biology research effort established at CPR is now the strongest at the University of Copenhagen and has contributed to the field by paving novel strategies for incorporating medical terminologies and ontologies in the integrative network biology analysis.

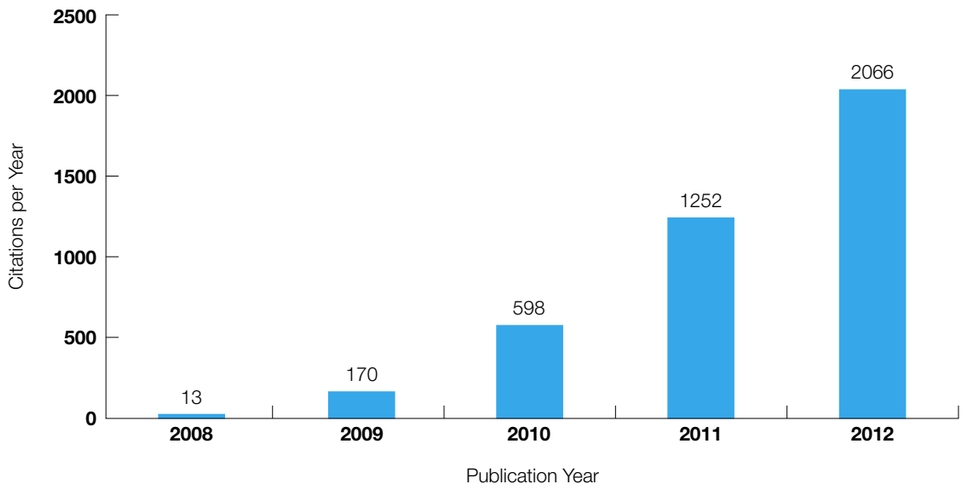
That the scientists at CPR have managed to perform world-class science is witnessed by the more than 150 peer-reviewed published manuscripts, of which more than twenty appeared in absolute top journals such as Science, Nature and Cell. This accomplishment should furthermore be judged in relation to the fact that CPR was established from scratch and the first two years of its existence were largely devoted to building activities and establishing the research infrastructure.

Scientific Output 2008-2012

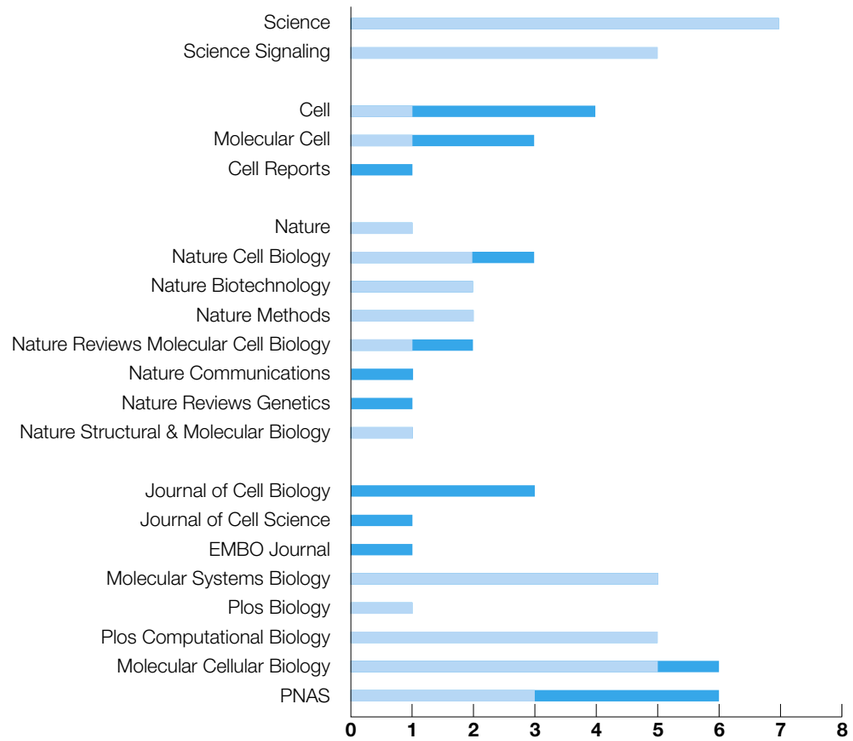
In 2012 CPR continued to be highly productive with regard to published papers in peer-reviewed journals, as summarized in the figures below. More than 70 scientific papers were accepted in a number of high-impact journals including Cell, Molecular Cell, Nature Structural & Molecular Biology, Nature Cell Biology and Nature Reviews. According to the ISI Web of Knowledge, more than 60 publications authored or co-authored by CPR scientists were published in 2012.



Number of publications authored or co-authored by CPR scientists (ISI Web of Knowledge).



Citation count per year of publications co-authored by CPR researchers (ISI Web of Knowledge).



CPR publications in high-profile journals.

Department of Disease Biology

With the arrival of Jiri Lukas, the Department of Disease Biology is now fully staffed and consists of five research groups. The Department of Disease Biology complements the CPR setup by focusing on 'physiological endpoints' of protein research, namely mechanistic dissection of medically relevant protein modifications and signaling pathways. The integration of the Lukas group is a significant addition to the research scope, both analytically and conceptually. The Lukas group brings its pioneering expertise in combining genetic screens with high-content microscopy; a powerful approach to identify and functionally dissect protein pathways. The Lukas group also provides the unifying conceptual ramification for all other groups in the department through its emphasis on genome integrity-maintenance mechanisms and their malfunction in disease.

Chromosome Stability and Dynamics
Group Leader, Prof. **Jiri Lukas**
The Chromosome Stability and Dynamics group was inaugurated in June 2012 and has brought a complementary expertise in genome integrity maintenance and an extensive international scientific network. Apart from Jiri Lukas, the group comprises one associated professor, three postdocs, two technicians and two PhD students.

The main research focus of the group is proteins and signaling pathways that regulate chromosome stability during the cell division cycle and after DNA damage, with a particular emphasis on how these mechanisms are subverted in disease. The long-term research interests of the group include proteins and protein complexes involved in cell cycle regulation and cellular responses to genotoxic stress; the research fields where Jiri Lukas has made a significant impact during the past two decades. The key scientific objectives of the group revolve around a nuclear compartment generated by DNA damage and often dubbed as a 'repair focus'. Repair foci reflect an exquisitely complex, hierarchical, and dynamic congregation of DNA-associated and histone-associated proteins and protein modifications. Highly coordinated action of these so-called 'genome caretakers' guards against chromosomal aberrations that could predispose to diseases such as cancer. The Lukas group has contributed to this field in many ways, including the recent study published in Cell, which is the first paper from the group to be finalized during Jiri Lukas' service as CPR Executive Director. The study includes collaborations with established CPR scientists and it represents one of the CPR milestones in 2012. It is described in detail in the Research Highlights 2012 section.

Since his arrival at the CPR, Jiri Lukas has been elected as a member of the Royal Danish Academy of Sciences and Letters. Claudia Lukas, a senior scientist and an associated professor in the Lukas group, has been awarded DKK 1.5 million from the Novo Nordisk Foundation. Additional financial support transferred to the CPR includes funds from the Danish Cancer Society, the Danish Council for Independent Research, the Danish National Research Foundation, and EMBO.

The group has several in-house collaborative projects with the Olsen group (on ATR phospho-proteome and its function during replicative stress) and the Mailand group (on structural and functional dissection of ubiquitin-regulated genome surveillance pathways), as well as external collaborative projects with the Advanced Light Microscopy Facility, EMBL, Heidelberg, Germany (Drs. Jan Ellenberg, Rainer Pepperkok, Beate Neumann, and Jean-Karim Heriche), the Center for Healthy Aging, Faculty of Health and Medical Sciences, University of Copenhagen (Ian Hickson), the Danish Cancer Society (Jiri Bartek), NIH/NCI, Bethesda, US, (Andre Nussenzweig), and CNIO, Madrid, Spain (Oskar Fernandez-Capetillo).

Chromatin Structure and Function
Group Leader, Assoc. Prof. **Jeremy Daniel**
The Daniel group initiated activities in January and currently has two postdocs and a student helper. The group covers another outstanding area in the genome integrity field, namely how DNA repair pathways operate within the context of chromatin and how malfunction of these mechanisms contributes to cancer development.

The specific research aim of the group is to elucidate how protein complexes that modify or associate with chromatin, promote DNA double-strand break repair and prevent lymphoid malignancy. DNA double-strand breaks (DSBs) can occur from exposure to genotoxic chemicals or irradiation, or as a result of collapsed replication forks. They also occur in lymphocytes as part of normal physiology to generate antibody diversity. The Daniel group's research program aims to address the following outstanding questions in the field:

1. How does PTIP mediate its critical function to generate antibody diversity in B-lymphocytes?
2. What role does lysine acetylation play in DNA recombination events occurring in lymphocytes?
3. Why is having kinase-dead ATM much more severe than having complete loss of ATM protein during embryonic development?

Answering these questions for lymphocytes and embryonic stem cells is expected to have broad implications for the roles of post-translational modifications in maintaining genome stability.

A great effort by the Daniel group went into building up the laboratory. Yet, the group still succeeded in delivering important scientific results in a study published in J Cell Biol. In this study, they demonstrated a previously unknown essential role for ATM kinase activity during embryonic development. The study is described in more detail in the Research Highlights 2012 section.

Jeremy Daniel recently received a Sapere Aude Starting Grant of approximately DKK 7 million from the Danish Council for Independent Research for his project proposal entitled Specificity of PTIP Complex Function in B-lymphocytes. A postdoctoral fellow in the group, Linda Starnes, was awarded her own postdoctoral fellowship funding from the Danish Independent Research Council for her project proposal entitled Elucidating the Function of ATM Kinase Activity during Development.

The Daniel group has initiated collaborations with the Department of Proteomics within CPR as well as with Dr. Joshua Brickman's group at another center funded by the Novo Nordisk Foundation; the Danish Stem Cell Center (DanStem).

Mitotic Mechanisms and Regulation
Group Leader, Assoc. Prof. **Jakob Nilsson**
The Nilsson group presently comprises four postdocs, three PhD students and one master student.

The group focuses on understanding how proper segregation of chromosomes is achieved during cell division with special emphasis on the Spindle Assembly Checkpoint (SAC). Failure in this process is a hallmark of cancer cells and contributes to tumorigenesis. Our activities are strongly integrated with the group of Jesper V. Olsen to identify novel interactors and post-translational modifications (PTMs) regulating chromosome segregation and with the Protein Production Facility (PPF) at CPR, to verify these by biophysical approaches using recombinant proteins.

The group has made significant progress in 2012 elucidating how substrates of the Anaphase Promoting Complex, a large ubiquitin ligase, are degraded in a temporal manner during mitosis (63). This study will be described further in the Research Highlights 2012 section. Furthermore, they have uncovered a novel interaction between the checkpoint protein BubR1 and the B56-PP2A phosphatase complex and shown that this is essential for proper chromosome segregation (34).

Molecular Endocrinology
Group Leader, Prof. **Amilcar Flores Morales**
The group comprises three postdocs, one research assistant and one PhD student.

The research goal of the group is to increase our understanding of the signaling mechanisms responsible for the initiation and progression of prostate cancer in order to identify regulatory proteins and pathways that can be targeted for prevention or treatment. To this end, the group is using genomic and proteomic profiling to study prostate cancer cells, animal models and clinical samples. Given the clear endocrine dependency of these tumors, the group is especially interested in understanding how steroid hormones interact with cytokines and growth factors to regulate prostate cancer progression.

During 2012 the group contributed with two important papers revealing the role of SOCS2 ubiquitin ligase in regulation of the systemic response to high-fat diets. The group has demonstrated that SOCS2 acts as a ubiquitin ligase for the growth hormone receptor and identified SOCS2 as an important regulator of hepatic homeostasis under conditions of high-fat dietary stress. The tumor-suppression functions of SOCS2 in prostate cancer have also been analyzed. These studies suggest that growth hormone receptor antagonists already in clinical use may be useful in treating metabolic alterations associated with obesity or systemic inflammation, and may have activity in a subset of patients with advanced prostate cancer. In collaboration with scientists at the Department of Proteomics and with clinical scientists from Umeå University, we have finalized the first large-scale quantitative proteomic profiling of prostate cancer clinical samples. This unique dataset will allow us to identify pathways that constitute disease aggressiveness.

Ubiquitin Signaling Group
Group Leader, Prof. **Niels Mailand**
The group currently has 17 members, including one professor, three associate professors, five postdocs, and eight PhD students.

The main focus of the group is to identify and characterize cellular signaling processes mediated by ubiquitin and ubiquitin-like modifier proteins, which function in genome stability maintenance and innate immunity. Current research in the lab fits broadly into five complementary and interconnected themes:

1. Identification of novel ubiquitin-dependent and SUMO-dependent signaling processes in the DNA damage response
2. Molecular regulation of translesion DNA synthesis
3. Novel factors and ubiquitylation targets in cellular response to DNA double-strand breaks
4. Biophysical and biological characterization of new SUMO-binding domains
5. Functions of ubiquitin signaling in innate immunity

The Mailand group identified a novel human protein, DVC1 (C1orf124), which plays a key role in facilitating ubiquitin-dependent responses to replication stress. In collaboration with the Lukas group, they discovered a novel ubiquitin ligase, RNF169, which, unexpectedly, restrains rather than promotes the ubiquitin-dependent response to DSBs. The group also provided new insight into the regulatory complexity of how ubiquitylation and SUMOylation cooperate to orchestrate protein interactions with DSB repair foci. Finally, in a recent joint study, the Mailand and Choudhary groups reported the first proteome-wide survey of DNA damage-regulated site-specific ubiquitylation in human cells. This study brings unprecedented insight into the extent to which ubiquitin operates as a signaling mechanism in cellular responses to DNA damage; knowledge that is essential for understanding the molecular basis of a range of human malignancies resulting from genome alterations. The mentioned studies are described in further detail in the Research Highlights 2012 section.

The group has raised more than DKK 30 million in external funding, and in 2012 additional external grants were awarded from: the Danish Cancer Society, the Lundbeck Foundation, the Novo Nordisk Foundation, and the EU (Marie Curie Fellowship (FP7)).

Protein Function and Interactions

Group Leader, Assoc. Prof. **Mats Wikström**

The research of the group is focused on understanding molecular recognition through biophysical and structural studies of protein-protein interactions applied to systems of biomedical importance.

In collaboration with Niels Mailand (Ubiquitin Signaling group), the Wikström group expressed in *E. coli* the novel ZZ SUMO-interacting domain derived from the HERC2 ubiquitin ligase and validated the interaction by biophysical methods. As an extension, this productive collaboration has identified similar ZZ domains in other proteins (CBP and MIB1, respectively) and a dedicated structural analysis of this novel interaction domain by NMR spectroscopy and X-ray crystallography is currently underway.

A major effort in the group is dedicated to a collaborative (and highly disease-relevant) project with Johan Malmström and Lars Björck (Lund University, Sweden) aimed at detailed structural analysis of virulence factors secreted by the human pathogen *Streptococcus pyogenes*. The group is expressing these proteins in *E. coli* for biophysical/structural and mass spectrometric (MS) studies in which the bacterial proteins will be used as bait in order to identify interacting human host proteins. This will be followed by X-ray crystallography to determine the structures of the complexes between the human host proteins and the bacterial proteins.

The group has already determined a structure for one of the novel secreted proteins. The goal of this project is to provide new opportunities for therapeutic intervention or for vaccine development programs to reduce the global burden of the *S. pyogenes* disease.

Department of Disease Systems Biology

Research Director, Prof. **Søren Brunak**

The department is led by Prof. Søren Brunak and Prof. Lars Juhl Jensen and consists of one associate professor, three postdoctoral fellows, nine PhD students and one Master student. The department consists of two research groups: Cellular Network Biology led by Lars Juhl Jensen, and Translational Disease Systems Biology led by Søren Brunak. There is also a research activity on Human Proteome Variation coordinated by Kasper Lage.

The key focus of the Department of Disease Systems Biology is to utilize computational biology for analysis of a wide range of data related to biology and medicine, and to broaden an already cross-disciplinary field into what has traditionally been the domain of 'medical informatics'. We have incorporated medical terminologies and ontologies in integrative network biology analysis in novel ways. Similarly, we have embarked on extracting adverse drug reactions from medical records and extended our work into the pharmacological and chemo-informatics areas.

The department has established an in-house platform for the analysis of post-translational modifications determined by mass spectrometry. This enables us to map efficiently modified peptides to genome data and thereby:

1. Study the structural context of the modifications (e.g. secondary structure and intrinsic disorder)
2. Compare modifications with each other (e.g. lysine acetylation and ubiquitylation)
3. Analyze the evolutionary conservation of modifications

This infrastructure has been used to analyze datasets from all three groups in the Department of Proteomics at CPR.

During 2012, the Brunak and Jensen groups developed an approach for rational selection of a subset of the kinome for screening by comparing all large-scale kinase inhibitor screens and quantifying the agreement between different types of binding assays between binding-based and activity-based assays. The new approach shows that it is possible to assess the selectivity of a compound for up to 88% of the kinome by only screening 100 kinases. Special attention has been paid to anti-neoplastic drugs and the method has been made available as an interactive web-based tool.

The Jensen group's research on cell-cycle regulation is an example of how computational analysis of already published data alone can lead to a major biological discovery. By correlating data on mRNA and protein levels during the mitotic cell cycle of several organisms with the codon usage of each gene, a phase-specific codon preference was discovered. This implies a hitherto unknown layer of regulation: that changes in the tRNA pool during the cell cycle affect translation rates in a transcript-specific manner.

One of the high impact activities of the Jensen group comes through providing web-based resources that enable wet-lab biologists to make use of state-of-the-art computational methods. The STRING database of protein-protein associations is a prime example of this; every week more than 5,000 different researchers submit in total more than 500,000 queries to the database. The group has also developed more specialized tools for analysis of phospho-proteomics data. So far these tools have only been used for in-house collaborations, but they will soon be made available to the community. Last but not least, the group has played a leading role in making text-mining technology accessible and usable by biomedical researchers.

The department currently includes individuals with diverse bioinformatics backgrounds, as well as pharmacologists, MDs and medical informaticists. In an era that is confronted with data from whole genome sequencing of populations and the emerging clinical proteomics efforts, this type of ultra-cross disciplinary work is what is needed, in particular if activities are to have translational ramifications.

Department of Proteomics

Research Director, Prof. **Matthias Mann**

The Department of Proteomics consists of three groups (Jesper V. Olsen, Chuna Ram Choudhary, and Michael L. Nielsen), which each have established their own lines of research, and Research Director Matthias Mann. The research groups have set up a world-leading biological mass spectrometry laboratory with state-of-the-art instrumentation, which includes six of the latest generation of fast sequencing, high-resolution Orbitrap tandem mass spectrometers. As proteomics is defined by its technological capabilities, one of the main objectives for the department is to continuously develop the integrated proteomics platform. The overall biological focus of the department is the global analysis of post-translational modifications (PTMs) of proteins in health and disease. The shared infrastructure has made the three independent groups highly efficient, while allowing each group to focus on their unique interests. The three lines of research are: Mass Spectrometry for Quantitative Proteomics, Proteomics and Cell Signaling and Proteomics Technology Development and Application.

Grants and Awards:

Mathias Mann was awarded three prizes in 2012 for his development of methods to analyze proteins using mass spectrometry. First, he received the Gottfried Wilhelm Leibniz Prize 2012, then he was awarded the prestigious 2012 Louis-Jeantet Prize for Medicine, and finally he won the prestigious Körber European Science Prize 2012.

Chuna Ram Choudhary was awarded the prestigious Sapere Aude grant from the Danish Council for Independent Research for his research focusing on proteomic analysis of immune and inflammatory signaling.

Michael Lundby Nielsen was granted DKK 1.2 million from the Lundbeck Foundation for his project on Specificity and Commonality of the Phosphoinositide-binding Proteome Analyzed by Quantitative Mass Spectrometry.

Jesper Velgaard Olsen was granted one fully financed PhD student fellowship as part of the Marie Curie International Training Network 'UPStream' on ubiquitin signaling and one EMBO postdoctoral fellowship for Dr. Chiara Francavilla for research on receptor tyrosine kinase trafficking.

Mass Spectrometry for Quantitative Proteomics

Group Leader, Prof. **Jesper Velgaard Olsen**

The Olsen group currently consists of three postdoctoral fellows, three PhD students and one Master student as well as two Novo Nordisk STAR postdoctoral fellows in collaboration with the Hagedorn Research Institute and one industrial PhD student shared with Symphogen A/S.

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 Olsen group is applying the developed proteomics technology to systems-wide analyses of dynamic post-translational modifications (PTMs) of proteins such as phosphorylation, ubiquitination, acetylation and glycosylation. These PTMs regulate cell signal transduction pathways. In particular, the group has developed the phosphor-proteomics technology that is now applied in many laboratories around the world.

In 2012 the group focused on developing proteomics methods to analyze PTMs in tissue samples from rodent models as well as human muscle biopsies. In an inter-departmental collaboration with Disease Systems Biology the Olsen group used high-resolution mass spectrometry to generate the broadest tissue catalog of phosphorylated and lysine acetylated proteins to date. The group localized modification sites to specific amino acids and showed that a number of these modifications function as cellular switches of enzymatic activity.

In total, the Olsen group published 11 peer-reviewed research papers during 2012, including publications in top journals such as Science Signaling, Nature Communications, Molecular Cell, PNAS and Cell Reports. These investigations were performed in close collaboration with external partners such as Eske Willerslev, and Thue Schwartz (University of Copenhagen), and Alfred Vertegaal (Leiden University, NL).

Proteomics and Cell Signaling

Group Leader, Assoc. Prof. **Chuna Ram Choudhary**

The group consists of five postdoctoral researchers, one graduate student, and one bachelor student.

The Choudhary group is interested in the proteome-wide investigations of PTM dynamics, with special focus on lysine acetylation and ubiquitylation, in response to various cellular perturbations and biological processes.

The group has made significant contributions to portraying a detailed picture of the regulatory landscape of acetylation and ubiquitylation in human cells by identifying thousands of novel PTM sites, and by uncovering their novel regulatory roles for selected proteins. The group has also initiated new lines of research to investigate the cross-talk between lysine acetylation and ubiquitylation. In 2011, the group developed a new MS-based method for ubiquitylation analysis which, for the first time, allowed unbiased and site-selective quantification of ubiquitylation on a global scale. The validity of this method was further demonstrated in murine models revealing diverse ubiquitylation patterns in different tissues (70). The group has collaborated with scientists at CPR as well as international researchers to unravel the complexity of phosphorylation, acetylation, and ubiquitylation networks that function in DNA damage response signaling (7, 58).

The group is collaborating extensively with researchers at national and international levels, including Stephen P. Jackson (University of Cambridge), Ivan Dikic (University of Frankfurt), Ian Hickson and Michael Lisby (University of Copenhagen), Niels Mailand, Jesper V. Olsen, and Michael L. Nielsen (CPR).

The group received a Sapere Aude Starting Grant and a postdoctoral research grant totaling more than DKK 8.5 million.

Proteomics Technology Development and Application

Group Leader, Assoc. Prof. **Michael Lund Nielsen**

The Nielsen group currently consists of three postdoctoral candidates, two PhD students and one Master student. The group is focused on a continuous development of the sensitive mass spectrometry (MS)-based platform available at the Department of Proteomics. The group has made a strong scientific impact in various biological areas, including the field of ubiquitination, in close collaboration with other groups at CPR (56, 58 and 70). The established proteomics platform at CPR now enables identification of thousands of post-translational modifications (PTMs), and the group aims at developing novel technologies for investigation of currently not-so-well-characterized PTMs. Furthermore, the Nielsen group is a key player in the EURAtrans EU FP7 consortia grant, aiming at utilizing next-generation sequencing technologies to generate genomic, transcriptomic and epigenomic datasets to identify the major functional pathways underlying human inflammatory, cardiovascular and metabolic, and behavioral disorders.

During 2012, the Nielsen group made considerable progress within proteomics technology development and their application in various biological areas. One of the proteomic technologies developed by the Nielsen group describes a fast and efficient methodology for protein digestion and mapping protein-protein interactions, and this is a convenient high-throughput methodology for affinity-purification mass spectrometry (AP-MS) experiments. Using the method developed, it is feasible to carry out rapid AP-MS experiments by shortening the time required for sample preparation and MS analysis from a few days to just a few hours (55). Moreover, in collaboration with the Olsen group, the Nielsen group has developed new MS strategies for optimal performance of the Q Exactive (32), and utilized this improved strategy to perform comprehensive profiling of proteome changes on sequential deletion of deubiquitylating enzymes (56). Our analysis supports that DUBs induce both direct and indirect effects on protein abundance, and that removing just a single component from the complex ubiquitin system causes major changes in cellular protein expression.

Protein Production Facility

Head, Assoc. Prof. **Mats Wikström**

As part of the transition to the second funding period, the former Department of Protein Science and Technology (PST) has been reconfigured into new and more focused units represented by the Protein Production Facility (PPF) and Chemical Screening Micro-node, respectively. These re-arrangements aim at better integrating this unit to the ambitious scientific plans and the salient features of the new structure can be outlined as follows:

The state-of-the-art protein production is a cornerstone of a competitive protein research center and new PPF has been carefully designed to reflect the current development in the field and meet the increasing demands. The main mission of this unit is innovative protein production as a tool for competitive basic and translational protein research. Among other benefits, this opens up a possibility to develop a nexus for protein production that would integrate groups within the CPR and other research centers funded by the Novo Nordisk Foundation. The PPF operates according to the following principles:

The Facility is headed by Mats Wikström (one of the previous leaders of the PST with a widely respected authority among its staff, and strong scientific track record in protein biochemistry, biophysics). In addition, Mats Wikström currently supervises all structural biology projects, in close coordination with the PPF.

The Facility is highly project-oriented and focused on innovative approaches such as difficult proteins and protein modifications, coupled to superior purity and functional characterization.

The Facility primarily targets ‘strategic research customers’ (CPR and other Novo Nordisk Foundation centers) and, whenever the capacity permits, also competitive Danish and international groups.

The Facility accommodates a core staff of 12 people (team-leaders, researchers and technicians) covering protein production, protein purification, and biophysical and functional protein characterization.

As a core facility, the PPF does not directly supervise PhD projects but it does directly participate in research projects in other CPR departments and contributes to the educational portfolio by organizing practical courses in the area of protein production and characterization.

Chemical Screening Micro-node

Following the strategy to gain maximum advantage of the existing technology and increase the outreach of the CPR to the Faculty of Health and Medical Sciences, the former chemical screening unit has been transformed into a CPR ‘micro-node’, a joint venture between the Costerson Biofilm Center (CBC), headed by Prof. Michael Givskov at the Department of International Health, Immunology and Microbiology Faculty, with whom CPR already collaborated on projects involving chemical biology screening.

The chemical screening platform has been physically transferred to the CBC (located at the Panum campus close to CPR) but continues to be owned by CPR. Such arrangement significantly broadens integration of CPR into the Faculty of Health and Medical Sciences, and opens up possibilities for the Faculty structures to benefit from the chemoinformatic activities directly at CPR.

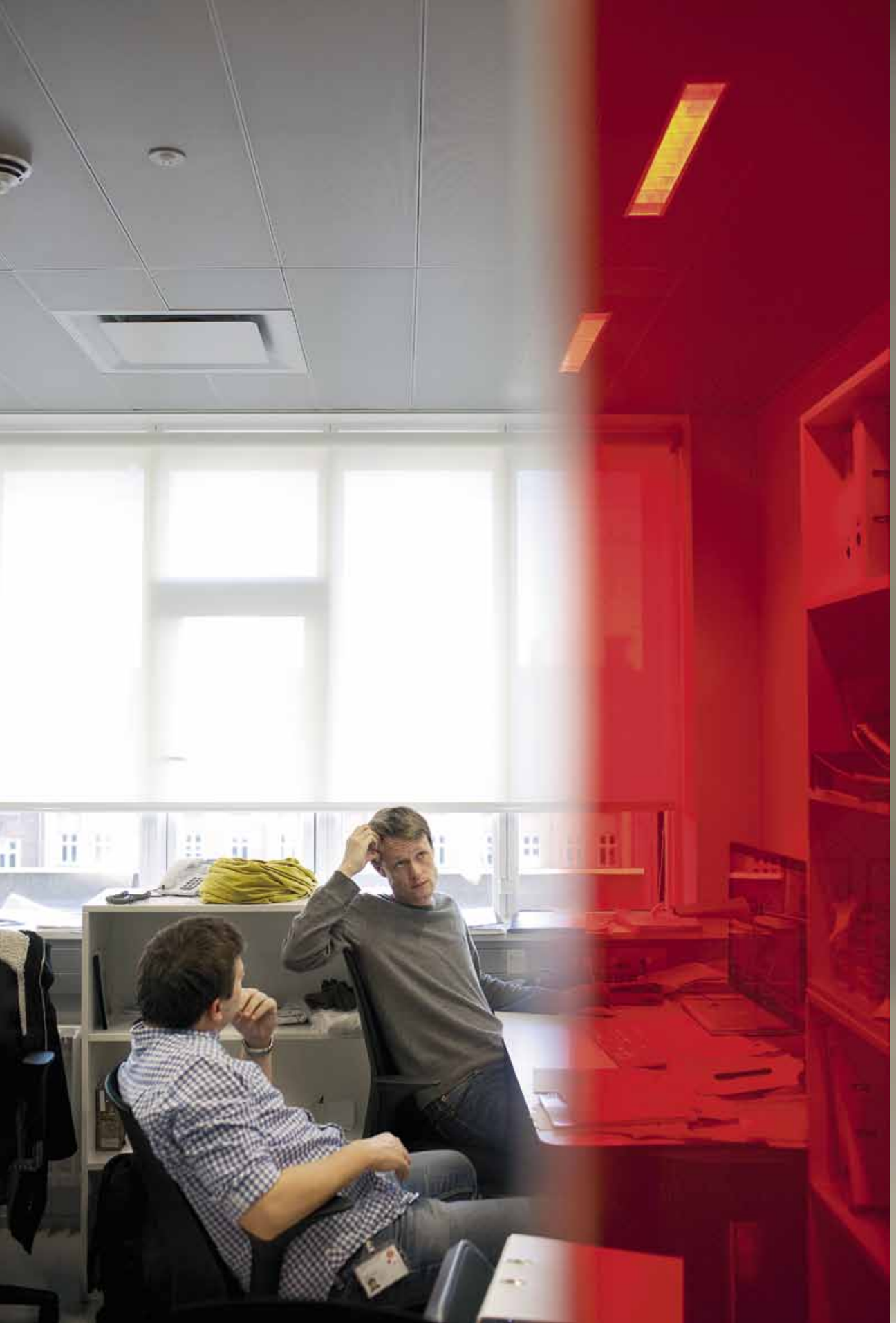
The screening facility is operated by Jens Berthelsen, an experienced chemical biologist, who originally set up this technology at the PST department and who has been formally transferred to the CBC.

Center Administration

Recruitment of New Center Administrator

Head of Administration **Peter Dyrsting**

Peter Dyrsting, cand.merc. from Copenhagen Business School, former Finance and Planning Manager at the Department of Odontology (School of Dentistry) at the Faculty of Health and Medical Sciences, accepted the position as Finance and Planning Manager by April 1st, 2012. Peter has many years of experience as an administrator both at University of Copenhagen and at the Technical University of Denmark, and he is considered one of the very best administrators at the Faculty. He has a very good strategic overview, experience within the various administrative areas, and a good knowledge of the University’s administrative procedures, and has helped to initiate and drive major changes in the School of Dentistry. We are happy to have Peter in charge of the CPR administration and he immediately proved to be a true professional with a great degree of independence, leadership skills, sensible judgment, and pleasant personality capable to generate the much needed spirit of shared commitment. His role in coordinating the CPR administrative matters is essential for the CPR scientific leaders to focus on research.



Research Highlights 2012

Revealing the Ubiquitylation Dynamics in Immunology and Genome Integrity Maintenance

A small modifier protein, ubiquitin, has emerged as a key regulatory factor in many critical DNA-damage signaling and repair pathways. However, our insight into the nature and scope of ubiquitin functions in the DNA damage response is still limited. During 2012, CPR has contributed with several studies unraveling the ubiquitylation mechanism.

Global Ubiquitylation Dynamics in Response to DNA damage

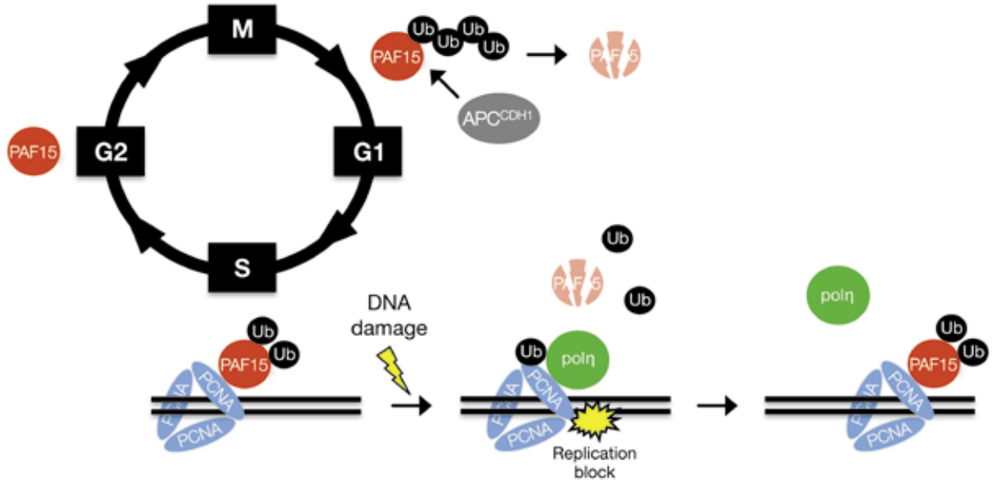
In a joint study published in Nature Cell Biology, the Mailand and Choudhary groups reported the first proteome-wide analysis of DNA damage-regulated ubiquitylation in human cells, revealing extensive involvement of ubiquitin-dependent protein modifications in cellular signaling pathways that protect genome integrity in our cells (58). The study brings unprecedented new insight into the extent to which ubiquitin operates as a signaling mechanism in cellular responses to DNA damage, and opens up many new avenues for the continued exploration of the roles of ubiquitin in safeguarding genome stability. This in turn is of key importance for understanding the molecular basis of a range of human malignancies resulting from genome alterations. From their global survey of DNA damage-regulated ubiquitylation, the authors also established a critical function of DNA damage-regulated ubiquitylation of the PAF15 protein in promoting DNA damage bypass, an important cellular means of preventing gross chromosomal instability.

Discovery of a New Human Protein, DVC1, Crucial for Protection of Genome Stability

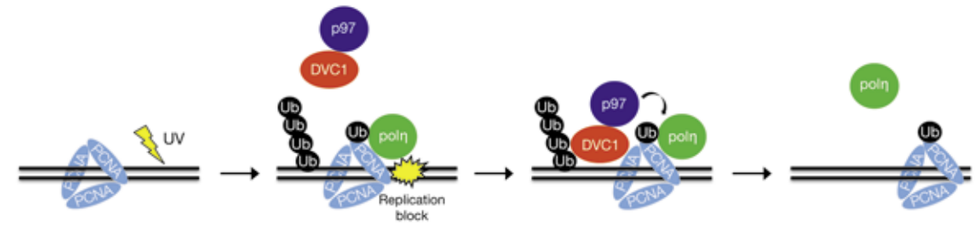
To mitigate the DSBs, cells have evolved damage-avoidance strategies to enable bypass of replication-blocking lesions. The predominant damage-bypass mechanism in mammalian cells involves specialized low-fidelity translesion synthesis (TLS) DNA polymerases, which are able to replicate damaged DNA and thus allow the replication machinery to bypass the lesion. Ubiquitin is known to play a central role in coordinating the TLS process with normal DNA replication, but our understanding of this involvement is still limited.

In collaboration with the Lukas and Choudhary groups, the Mailand group has now identified an uncharacterized human protein, DVC1 (C1orf124), as the first adaptor protein for the ubiquitin-selective chaperone VCP/p97 that specifically targets it to sites of DNA damage. Through such DVC1-mediated targeting, p97 promotes important ubiquitin-dependent transactions at blocked replication forks, including the displacement of the TLS polymerase polη to preserve overall genomic integrity (46). In collaboration with the Pocock group at the Biotech Research and Innovation Centre (BRIC), we also showed that the DVC1 function is highly evolutionarily conserved from *C. elegans* worms to humans.

These findings establish DVC1 as a DNA damage-targeting p97 adaptor that protects cells from the deleterious consequences of replication blocks, and provide important new mechanistic insight into how ubiquitin-mediated processes regulate cellular responses to DNA damage.



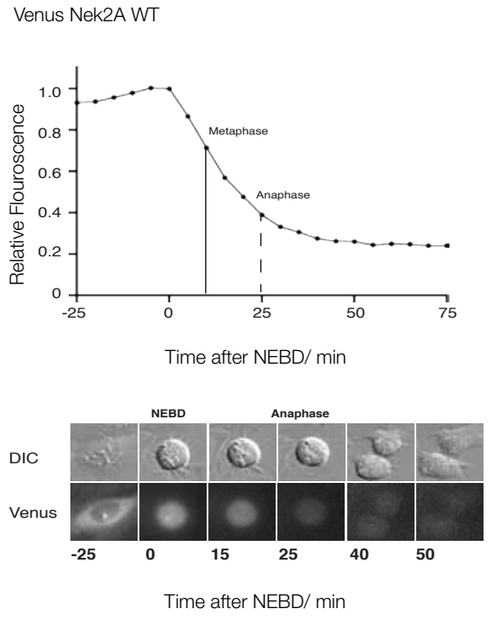
PAF15 functions as a gatekeeper for translesion DNA synthesis (TLS), an important yet mutagenic means of bypassing DNA lesions during DNA replication. DNA damage-induced degradation of PAF15 enables TLS polymerases to gain access to PCNA at the replication fork and replicate damaged DNA.



DVC1 promotes ubiquitin-dependent responses to replication blocks. DVC1-mediated recruitment of the ubiquitin-selective chaperone p97/VCP to stalled replication forks triggers displacement of TLS polymerases, limiting error-prone DNA replication.

Ubiquitylation and SUMOylation Events Cooperate to Regulate Protein Interactions with DSB Repair Sites

Ubiquitin and SUMO play interconnected roles in DSB-induced signaling and repair (reviewed in Bekker-Jensen *et al.* 2011). The Mailand group identified the DSB-responsive ubiquitin ligase HERC2 as an important target of DSB-induced SUMOylation. Via a novel type of SUMO-binding motif, the ZZ-type Zinc finger, DSB-associated HERC2 SUMOylation facilitates the activation of the DSB-induced RNF8/RNF168 ubiquitin ligase cascade that ubiquitylates DSB-flanking chromatin areas (16). These findings provide novel insight into the regulatory complexity of how ubiquitylation and SUMOylation cooperate to orchestrate protein interactions with DSB repair foci, and identify a new mechanism by which SUMOylation signals are decoded in cells.



The degradation of a substrate of the APC/C complex during mitosis followed in a live cell assay. The fluorescence intensity of the substrate is measured as the cell progresses through mitosis to determine when the degradation starts.

Insight into the Temporal Degradation of Proteins during Mitosis

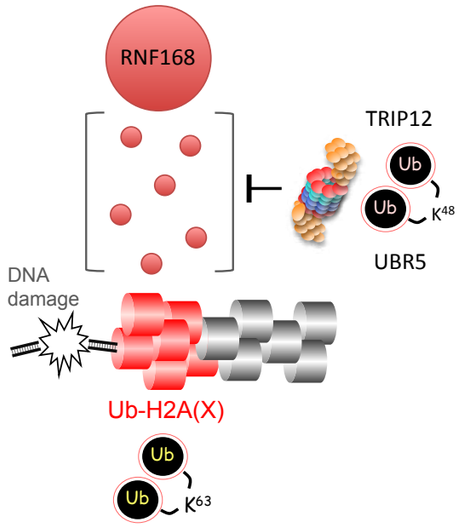
Orderly progression through mitosis is driven by the degradation of specific proteins at specific times. Proteins are targets for degradation by the large E3 ubiquitin ligase; the Anaphase Promoting Complex (APC/C), but exactly how this ligase achieves orderly temporal degradation has not been clear. In collaboration with groups in Cambridge, the Nilsson group has now provided insight into this by identifying and analyzing the degradation of substrates in real-time degradation assays performed directly in living cells. A striking correlation between affinity of the substrate for the APC/C and its timing of degradation was observed, explaining how the APC/C temporally selects its substrates in a 'soup' of proteins (63).

Discovery of Novel Ubiquitin Ligases Important for Genome Integrity Maintenance: TRIP12 and UBR5:

Histone ubiquitylation is prominent among modifications that cooperate to restore the integrity of DNA-damaged genome. It is triggered by a complex cascade of enzymatic reaction including RNF168, an E3 ubiquitin ligase that amplifies ubiquitin reactions specifically at damaged chromosomes. Although beneficial for DNA repair, it is crucial that this ubiquitinylation activity is confined to the site of the DNA lesion, leaving the undamaged chromosomes untouched. Otherwise, an excessive chromatin ubiquitylation and spreading away from the site of damage could have far-reaching deleterious consequences in the healthy parts of the genome.

In their new study, Jiri Lukas and his colleagues screened the human ubiquitinome for suppressors of RNF168, and identified two E3 ligases, TRIP12 and UBR5, that regulate RNF168 stability and thereby suppress excessive chromatin ubiquitylation in cells challenged by DNA breakage (23). Apart from uncovering a molecular pathway, which guards against

illegitimate spreading of repair-associated enzymatic reactions, this study has implications for cancer research by showing that hyperaccumulation of RNF168 is a frequent ‘molecular signature’ of cancers associated with oncogenic human papillomaviruses.

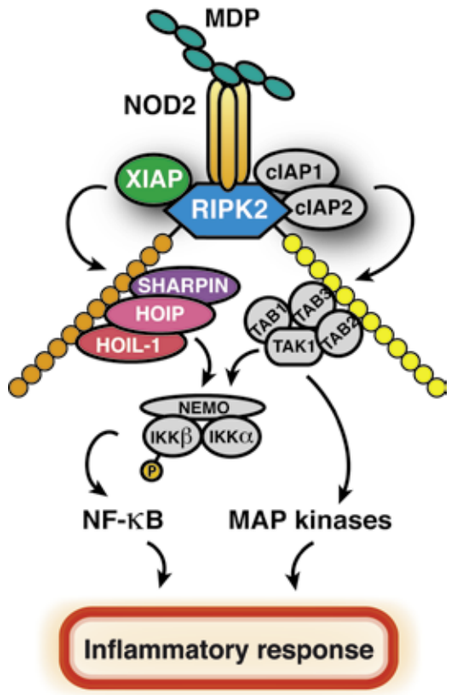


Molecular mechanisms that confines DNA damage-induced chromatin ubiquitylation to the proximity of DNA lesions and prevents spreading of this modification to the healthy parts of the genome.

Ubiquitylation Dynamics in Immunology

Defects in the gene encoding the XIAP protein cause a serious immune malfunction, called X-linked lymphoproliferative syndrome type 2 (XLP2), which affects male children.

Researchers from the Mailand group have used biochemical analyses to map how the XIAP protein activates a vital component of the immune defense system, specifically a component that fights bacterial infections in the gastro-intestinal system (13). Normally, the gastric intestinal tract is surrounded by an immune barrier that hinders the bacteria of the gut from spreading in the rest of the body. Patients with XLP2 syndrome suffer from an immunodeficiency that disrupts this barrier. The study shows that genetic mutations found in patients with XLP2 specifically destroy XIAP’s ability to attach the signaling protein ubiquitin to other proteins. The attachment process is vital for activating the immune system and therefore also for survival. The results constitute important steps towards gaining understanding of the very serious – and fortunately rare – genetic immune disorder as well as providing important knowledge for leukaemia research. Several pharmaceutical companies have developed drugs to act on IAP proteins, including XIAP, as part of cancer treatment. It is therefore essential to know precisely which biological processes in the organism the treatment can potentially affect. The work was done in close cooperation with Philipp Jost (Technisches Universität München, Germany), John Silke and Andreas Strasser (Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia) and Henning Walzcak (Imperial College, London, UK).



Recognition of bacterial peptidoglycans by cytosolic receptor NOD2 leads to activation of immune responses.

Tissue-specific Proteomics of PTMs

Post-translational modifications are implicated in a diverse variety of cellular processes spanning from proliferation and differentiation to apoptosis. Site-specific phosphorylation or acetylation events often function as molecular switches that either activate or inhibit protein activity, and they have been implicated in the pathophysiology of several severe diseases, such as cancer, diabetes, and neuropsychiatric disorders. To better understand disease fingerprints and to evaluate which medical treatment is most appropriate for a patient, it would be beneficial if the phosphoproteome and acetylome of tissue samples from patients could be analyzed. However, for such analyses to be feasible it is a prerequisite that reliable and comprehensive methods are developed.

New Proteomics Methods: Organ-wide Mapping of PTMs *in vivo*

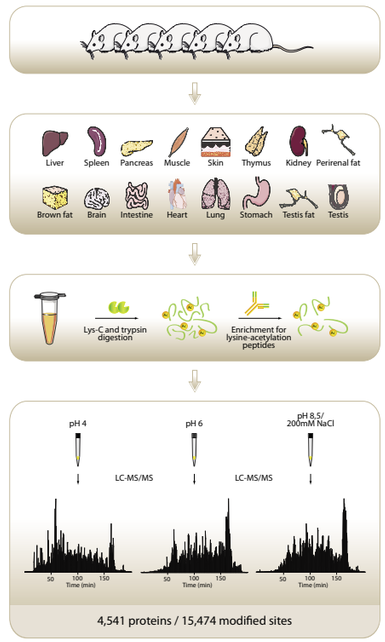
Based on the work from the Nielsen group, a sensitive mass spectrometry (MS)-based platform has been developed in the Department of Proteomics. The Nielsen group has made a strong scientific impact in various biological areas, including the field of ubiquitination, in close collaboration with other groups at CPR. The established proteomics platform at CPR now enables identification of thousands of ubiquitination sites and other PTMs such as phosphorylation, lysine acetylation, lysine hydroxylation, and arginine citrullination.

Organ-wide Maps of PTM Sites *in vivo*

In collaboration with the Department of Disease Systems Biology, members of the Olsen group used high-resolution tandem mass spectrometry to generate the broadest tissue catalog of phosphorylated proteins to date, and localized phosphorylation sites to specific amino acids. The generated dataset covered 31,480 phosphorylation sites on 7,280 proteins quantified across 14 rat organs and tissues and was made easily accessible to biologists via a web-based database, the CPR PTM Resource. As this method, developed by the CPR scientists, is much simpler and faster than previously reported methods, it provides a promising platform for standardizing screening of tissue phosphoproteomes (40).

In an analogous study focused on large-scale lysine acetylation, in collaboration with the Choudhary group, the Department of Disease Systems Biology and the Protein Production Facility, the Olsen group performed the first global analysis of lysine acetylation sites in tissue samples and quantified more than 15,000 sites across 16 different rat organs and tissues. It has long been speculated that the prevalence of lysine acetylation and its impact on cellular processes matches that of phosphorylation (39).

The dataset revealed that the sub-cellular acetylation distribution is tissue-type-dependent and that acetylation targets tissue-specific pathways involved in fundamental physiological processes. In the study the groups compared lysine acetylation patterns for rat as well as human skeletal muscle biopsies and demonstrated its general involvement in muscle contraction. Furthermore, the groups showed that site-specific lysine acetylation of the key metabolic enzymes fructose-bisphosphate aldolase and glycerol-3-phosphate dehydrogenase serves as a cellular mechanism to switch off their enzymatic activity, adding another layer of post-translational regulatory control to dynamic cellular processes.



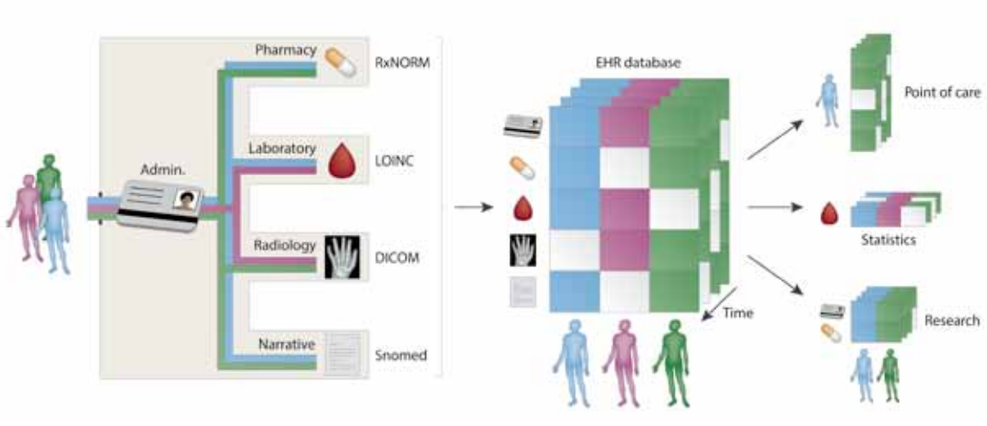
Proteomics workflow for large-scale analysis of lysine acetylation sites in rat tissues.

Finally, the Choudhary group has also initiated new lines of research to investigate the cross-talk between lysine acetylation and ubiquitylation. For this, the group developed a new MS-based method for ubiquitylation analysis which, for the first time, allows unbiased and site-selective quantification of ubiquitylation on a global scale. The Choudhary group found that only half of the ubiquitylation sites in human cells are increased after inhibition of the proteasome, suggesting that a substantial fraction of ubiquitylation sites may have non-proteasomal functions. The validity of this method was further demonstrated in murine models, revealing diverse ubiquitylation patterns in different tissues (70).

Mining Electronic Health Records: Towards Better Research Applications and Clinical Care

Clinical data describing the phenotypes and treatment of patients represents an under-used data source that has much greater research potential than is currently realized. Mining of Electronic Health Records (EHRs) has the potential for establishing new patient-stratification principles and for revealing unknown disease correlations. Integrating EHR data with genetic data will also improve understanding of genotype–phenotype relationships. However, a broad range of ethical, legal and technical reasons currently hinder the systematic deposition of these data in EHRs and their mining (26). In this respect Denmark has unique opportunities, as the availability and the legal situation are more favorable than in many other countries.

In a recent review published in Nature Reviews, the Department of Disease Systems Biology considers the potential for furthering medical research and clinical care using EHR data and the challenges that must be overcome before this is a reality. By integrating techniques from textmining, datamining and bioinformatics, the scientists performed statistical investigations of disease comorbidity, patient stratification and gene-trait association studies. Electronic Health Records (EHR/EPR) typically gather structured information about the individual patient in the form of administrative and demographic data as well as information about prescribed drugs, laboratory tests and results as well as imaging data and coded diagnostic and treatment data. In addition to this, unstructured data in the form of written narratives remain a cornerstone in clinical documentation. While this is typically the most abundant data type, it is also the most difficult to analyze computationally. However, there are significant benefits in terms of obtaining fine-grained phenotypes for patients from the unstructured data, for discriminating between treatment-related disease-disease correlations and those which are solely caused by germline or somatic genetic changes in the genome of the patient, and for discovering numerous other types of knowledge, such as novel adverse drug reactions for the 7,500 drugs approved in Denmark.

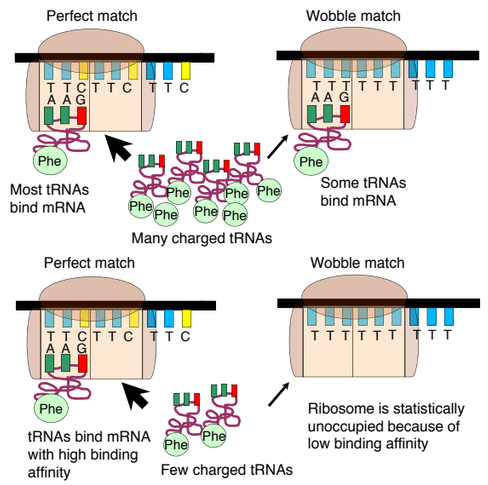


The Electronic Health Record (EHR) of a patient can be viewed as a repository of information regarding his or her health status in a computer-readable form. An encounter with the healthcare system generates various types of patient-linked data.

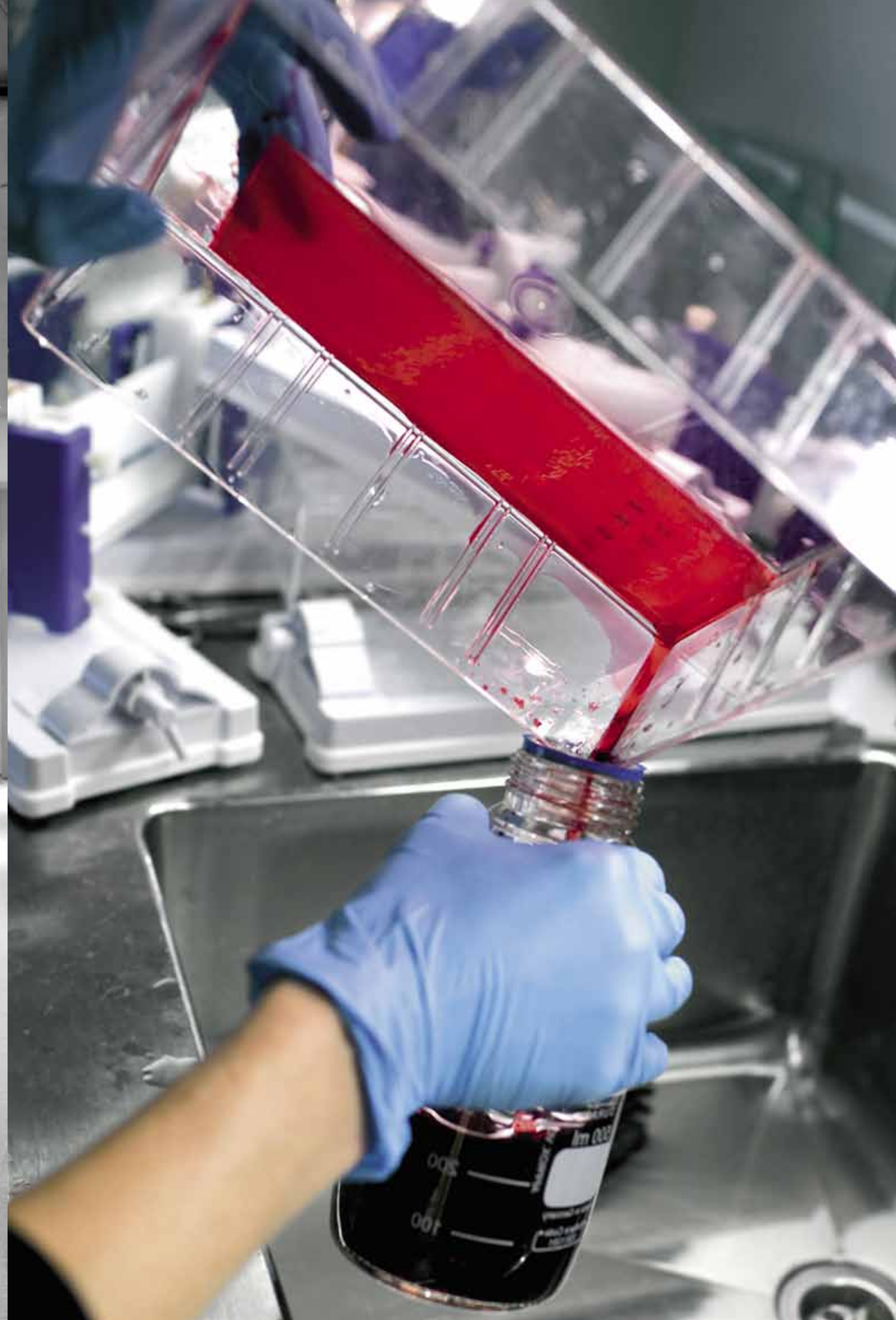
Less than Optimal: Codon Usage Analysis Adds another Layer to Cell Cycle Regulation

The cell-cycle is a temporal program that regulates DNA synthesis and cell division. In a highly international collaboration with researchers from the Weizmann Institute of Science in Israel, the Jensen group compared the codon usage of cell cycle-regulated genes with that of other genes, which led to the discovery that the former prefer non-optimal codons. Moreover, genes encoding proteins that cycle at the protein level exhibit non-optimal codon preferences, and cell cycle-regulated genes expressed in different phases of the cell cycle display different codon preferences.

Together with researchers from Thomas Jefferson University in Philadelphia, the Jensen group showed empirically that transfer RNA (tRNA) expression is indeed highest in the G2 phase of the cell cycle, consistent with the non-optimal codon usage of genes expressed at this time, and lowest toward the end of G1, reflecting the optimal codon usage of G1 genes. Accordingly, protein levels of human glycyl-, threonyl-, and glutamyl-prolyl tRNA synthetases were found to oscillate, peaking in G2/M phase. In light of these findings, Jensen and colleagues proposed that non-optimal (wobbly) matching codons influence protein synthesis during the cell cycle. Furthermore, they described a new mathematical model that shows how codon usage can give rise to cell cycle regulation. In summary, these results indicate that cells exploit codon wobbling to generate cell cycle-dependent dynamics of proteins (21).



A schematic presentation of the additional level of protein translation regulation via the tRNA pool. Top: The translation of poly-TTC and poly-TTT chains (used as an example) when the pool of charged tRNAs includes many TTC-tRNAPhe. Bottom: Changes in the translation rate of poly-TTC and poly-TTT chains if few TTC-tRNAPhe are available.



Training and Education

A major priority of CPR is to provide high-quality training for new generations of young scientists. Close to half of the staff at CPR are PhD students or postdoctoral fellows who receive support from highly qualified scientists and have access to advanced equipment and methods that allow them to work at an absolute top international level. In 2012, CPR housed 26 PhD students and 18 MSc/BSc students. The first CPR PhD student finished his education and defended his PhD thesis in 2012. Heiko Horn from the Department of Disease Systems Biology defended his thesis entitled: Post-translational Modification in Cell Signaling – Regulation and Deregulation.

During 2012 CPR organized two theoretical PhD courses combining lectures with journal clubs and student presentations, and CPR participated in one under-graduate course for human biologists, two EMBO practical courses, and a spring school for molecular biologists.

The PhD course, **Mass Spectrometry-based Proteomics and its Applications in Biology**, was arranged by the Department of Proteomics and included external speakers. The aim of this PhD course was to provide the students with:

1. An overview of the major high-end quantitative proteomics technologies with focus on stable isotope labeling and high-resolution mass spectrometry
2. An overview of the wealth of biological applications that quantitative proteomics screens can be applied in, including global proteome quantitation, PTM analysis and protein-protein interaction screens.

Local as well as external experts were teaching on this course, including Matthias Selbach, Max Delbrück Center for Molecular Medicine, Berlin, Marcus Krüger and Jürgen Cox, Max Planck Institute, Germany, Boris Macek, Proteom Centrum, Tübingen University, Germany, Blagoy Blagoev, Ole N. Jensen, Frank Kjeldsen and Jens Andersen, University of Southern Denmark, Michiel Vermeulen, University Medical Center, Utrecht, NL, and Tiziana Bonaldi, IFOM-IEO Campus, Italy.

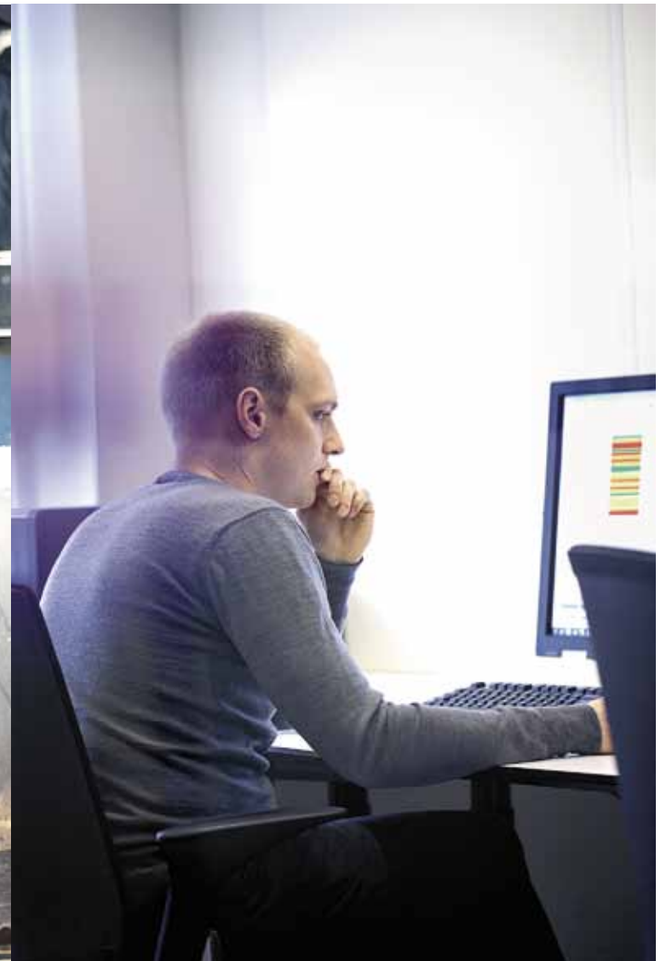
The PhD course, **Cellular Responses to DNA Damage**, was arranged by the Mailand group from the Department of Disease Biology. In this PhD course, the students were thoroughly introduced to this important and rapidly developing field in biological research. A range of lecturers taught the basic principles of the complex pathways that govern the DNA damage response. In addition, invited experts working within the field presented cutting-edge research on various aspects of the response. Specific emphasis was on the use of different model organisms and scientific tools, as well as the links to human genetic diseases. The course featured presentations from both local and external expert speakers including Uli Rass, Friedrich Miescher Institut, Basel, Switzerland, Lene Juel Rasmussen, Mansour Akbari and Ian Hickson, Center for Healthy Ageing, University of Copenhagen, Jurgen Marteijn, Erasmus Medical Center, Rotterdam, NL, Michael Lisby and Vibe Østergaard, University of Copenhagen, Claus Storgaard Sørensen, BRIC, University of Copenhagen, and Alan Lehmann, Molecular Genetics, University of Sussex, UK.

The University of Copenhagen undergraduate course, **Bioinformatics for Human Biologists**, was organized by the Department of Disease Systems Biology. The objective of the course was to introduce students to a range of new methods for computational analysis of biological sequences and structures and to put this in the context of modern health research. Computer-based methods play a decisive role in microbiology, biotechnology and pharmaceutical research, and major international sequence and structure databases contain information which may frequently replace or critically support experimental work. In other cases, the data serve to increase the yield of the experimental resources used. The course is part of the Molecular Biology and Genetics and Advanced Cell Biology course, offered to human biologist students.

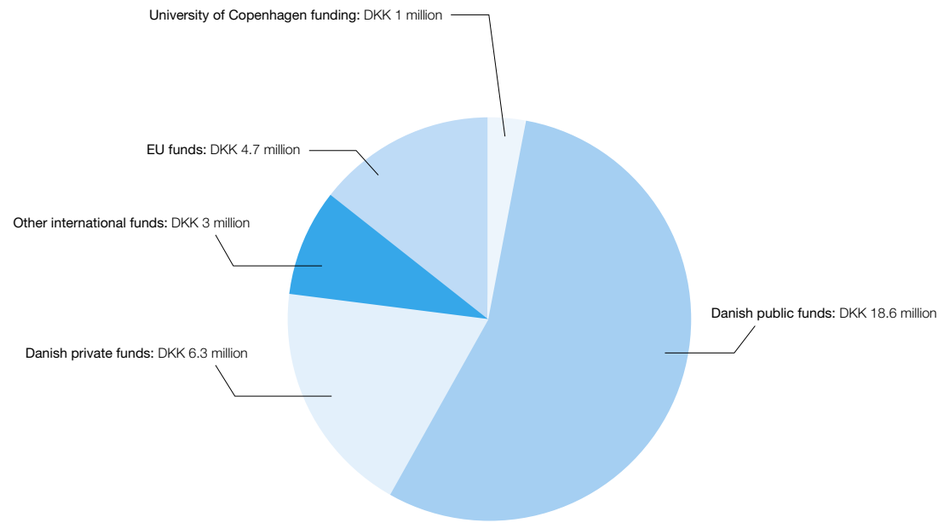
The Department of Disease Systems Biology also participated in teaching on the The European Molecular Biology Organization (EMBO) practical course, **Computational Analysis of Protein-Protein Interactions for Bench Biologists,** and the **12th Spring School of Bioinformatics for Molecular Biologists**. Both of these courses are introductory bioinformatics courses targeting graduate students and postdocs working in molecular biology laboratories. The courses teach students how and when to use an array of bioinformatics tools and also aim to give the participants a fundamental understanding of how to think like a computational biologist.

Finally, the EMBO practical course, **Computational Biology: From Genomes to Cells and Systems** was co-organized by Lars Juhl Jensen from the Department of Disease Systems Biology. The course focused on secondary data analysis, which is using published data sets in a novel manner rather than primary data analysis. It covered technical and strategic solutions to recurring problems in computational biology and provided the participants with a broad view of recent advances in the field. Lectures introduced the concepts and technologies such as next-generation sequencing and high-throughput proteomics, and tutorial sessions focused on the application of such data in particular in the context of a collaborative project on Crohn's disease. The teachers

on the course were Francesca Ciccarelli, European Institute of Oncology, Italy, Chris Creevey,Teagasc Animal & Grassland Research and Innovation Centre, Ireland, Konrad Förstner, University of Würzburg, Germany, Martijn Huynen Radboud, University Nijmegen Medical Centre, NL, Lars Juhl Jensen from CPR, Roland Krause, University of Luxembourg and Luxembourg Centre for Systems Biomedicine, Nuria Lopez-Bigas, ICREA and the University Pompeu Fabra, Spain, Christian von Mering, University of Zurich, Switzerland, and Jeroen Raes, Vrije Universiteit, Brussels, Belgium.



External Funding



Overview of the distribution of other external funds (DKK) granted in 2012 according to funding source.

2012 proved to be another successful year for attracting external funding to the Center. All in all, more than DKK 32.5 million (>EUR 4.3 million) was obtained in external funding. CPR has two new Sapere Aude: Starting Grant recipients (Danish Council for Independent Research), with this year's addition, Prof. Chuna Ram Choudhary, receiving DKK 7 million (EUR 0.9 million) his research project: Proteomic Investigation of Systemic Responses to Innate Immune and Inflammatory Cytokine Receptor Signaling, and Assoc. Prof. Jeremy Daniel, receiving DKK 7 million (EUR 0.9 million) for his research project entitled: Specificificity of PTIP Complex Function in B-lymphocytes.

CPR received three project grants from the Lundbeck Foundation: one of DKK 1.2 million (EUR 0.2 million) granted to Assoc. Prof. Michael Lundby Nielsen, Department of Proteomics, for his project: Specificity and Commonality of the Phosphoinositide-binding Proteome Analyzed by Quantitative Mass Spectrometry, one of DKK 1.5 million (EUR 0.2 million) granted to Simon Bekker-Jensen from Department of Disease Biology for his project: Structural and Functional Characterization of a Novel Class and SUMO-binding Domains, and finally a grant of DKK 1 million (EUR 0.13 million) for Assoc. Prof. Mads Gyrd-Hansen. Søren Brunak received DKK 2.2 million (EUR 0.3 million) for his project on beta-cell type I diabetes: Focus on Post-translational Modificationsfrom the Juvenile Diabetes Research Foundation (JDRF). Assoc. Prof. Claudia Lukas received DKK 1.3 million (EUR 0.2 million) from the Novo Nordisk Foundation for her project: Spatial-temporal Profiling of Genome Integrity Maintenance.

Finally, three postdoc grants from the European Molecular Biology Organization (EMBO) were transferred by Luis Ignacio Toledo Lazaro DKK 0.2 million (EUR 0.04 million) and Matthias Florian Altmeyer DKK 0.2 million (EUR 0.04 million) from the Department of Disease Biology, and by Chiara Francavilla DKK 0.3 million (EUR 0.04 million) from the Department of Proteomics. Linda Starnes from the Daniel group received a postdoc grant from the Danish Council for Independent Research, Medical Sciences of DKK 3 million (EUR 0.4 million). In addition, Petra Beli from Department of Proteomics received a postdoc grant also from the Danish Council for Independent Research of 1.5 million (EUR 0.2 million).

The Center is involved in eight EU FP7 collaborative projects. In 2012 CPR engaged as a partner in a Marie Curie International Training Network: UPStream, and a new collaborative project: BioMedBridges. The Marie Curie International Training Network UPStream is an EU-funded network grant for research training in the ubiquitin-proteasome system. The Olsen group at the Department of Proteomics is one of the partners in this network consisting of 11 European laboratories, all experts in ubiquitin biology, and was granted DKK 2.4 million (EUR 0.3 million) from the Danish Council for Independent Research. The project started in early 2012 and the main objective of the UPStream network is

training of the next generation of European scientists in a critical and complex field of modern biology, where the main purpose is the understanding of the regulation of the ubiquitin proteasome system and its potential use for drug development. One PhD student at CPR, Jón Otti Sigurðsson from the Department of Proteomics, is currently being funded by this grant and he is working full time on the project, giving him ample opportunities to interact with European experts in ubiquitin signaling and related areas. In addition to external funding, involvement in EU projects thus provides PhD students and postdoctoral fellows at the Center with easy and invaluable access to the elite in European research. Website: www.upstreamproject.eu

UPStream

The collaborative EU FP7 project, BioMedBridges, is a joint effort of ten biomedical sciences research infrastructures on the European Strategy Forum on Research Infrastructures (ESFRI) roadmap. The objective is to build data bridges between biological and medical infrastructures in Europe, co-coordinated by ELIXIR; Europe's leading life science organizations in managing and safeguarding data. Together, the project partners will develop the shared e-infrastructure – the technical bridges – to allow interoperability between data and services in the biological, medical, translational and clinical domains, and thus strengthen biomedical resources in Europe. The CPR/Department of Disease Systems Biology involvement in BioMedBridges concerns integrating disease-related molecular-level data (e.g. specific genes and proteins) and biomedical terminology, such as SNOMED-CT and ICD, for which they were granted DKK 2.4 million, (EUR 0.3 million). Website: www.biomedbridges.eu

BioMedBridges

At the CPR I am in the unique position to acquire advanced knowledge and methodological proficiency in several state-of-the-art technologies. At the same time I have great opportunity of establishing extensive collaborations with scientists from multidisciplinary backgrounds to advance my career and reach a position of professional maturity.

– Linda Starnes

Linda Starnes

Conferences Organized by CPR

The second Copenhagen Bioscience Conference sponsored by the Novo Nordisk Foundation and co-organized by CPR, focused on PTMs (post-translational modifications) in Cell Signaling. The organizers from CPR were Jesper V. Olsen, Lars J. Jensen, Chuna Ram Choudhary, and Niels Mailand.

Post-translational modifications in cell signaling have evolved as the eukaryotic cell's most important regulatory mechanism to regulate intracellular protein networks in response to external or internal cues. Site-specific modifications of proteins by PTMs are involved in virtually all signaling pathways that orchestrate fundamental cellular processes. Deregulation of proteins involved in important cellular responses can often lead to disease, and the enzymes catalyzing addition or removal of PTMs have therefore emerged as prominent therapeutic targets. The conference focused on the cellular regulation of these fundamental processes by bringing together world-leading researchers in the area of PTM functions in different cell-signaling networks.

The venue was the Comwell Borupgaard conference hotel at Snekkersten, north of Copenhagen, where 200 researchers from 27 different countries were accommodated for four intensive days. Since one of the main purposes of the Copenhagen Bioscience Conferences is to create a forum for exchanging knowledge and for promoting interaction between junior and senior researchers, the participants were evenly spread across all levels, from PhD fellows to professors.

Sixteen top researchers gave presentations on protein research including topics such as: autophagy, DNA-damage signaling, signaling systems and networks, metabolism and ageing, cell cycle as well as epigenetics and cancer signaling. Based on their applications and abstracts, 11 young participants were invited to give a brief lecture about their research.

During the conference, all attendees (from PhD fellows to professors) presented a poster, with a prize for the best PhD poster being awarded to Nicholas T. Hertz from the University of California (UCSF) and a prize for best postdoc poster being awarded to Berthe K. Fiil from CPR.

The conference's social program included a visit to the Tivoli Gardens in central Copenhagen, combined with a lecture by Nobel Laureate Peter Agre, Johns Hopkins University, on the subject How to OPEN DOORS with a Nobel Prize. Peter Agre was awarded the Nobel Prize for Chemistry in 2003. The Royal Danish Academy of Sciences and Letters co-arranged the event.

However, it might have been the magician Thomas Fraps who made the biggest impression. Pretending to be a slightly distracted protein researcher, he rounded off the scientific program with a lecture combining science and magic in a fascinating and very amusing way.

In the end, the conference was a great success in terms of drawing international awareness to Copenhagen as an important research hub, and more importantly in terms of creating new professional networks across disciplines and institutions and in providing new inspiration and knowledge to improve future research. The ambition of CPR is to keep this momentum and launch a regular bi-annual world-class forum to discuss the most novel discoveries and emerging paradigms in the role of PTMs in diverse areas of biology and medicine.

Speakers:

- **Peter Agre,** Nobel Prize Winner, Johns Hopkins University, US
- **Ivan Dikic,** Goethe University/FMLS, Germany
- **Steve Jackson,** Gurdon Institute, US
- **Jiri Lukas,** Novo Nordisk Foundation Center for Protein Research, Denmark
- **Andre Nussenzweig,** National Cancer Institute, US
- **Philippe Bastiaens,** Max Planck Institute of Molecular Physiology, Germany
- **Forest White,** Massachusetts Institute of Technology, US
- **Matthias Mann,** Max Planck Institute of Biochemistry, Germany
- **Eric Verdin,** University of California San Francisco, US
- **Søren Brunak,** The Novo Nordisk Foundation Center for Protein Research, Denmark
- **Brenda Schulman,** St. Jude Children's Research Hospital, US
- **Gerald W. Hart,** Johns Hopkins University, US
- **Michael B. Yaffe**, Massachusetts Institute of Technology, US
- **Henrik Semb,** DanStem, Denmark
- **Tim Hunt,** Cancer Research UK, UK
- **Angus Lamond,**The College of Life Science, University of Dundee, UK

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