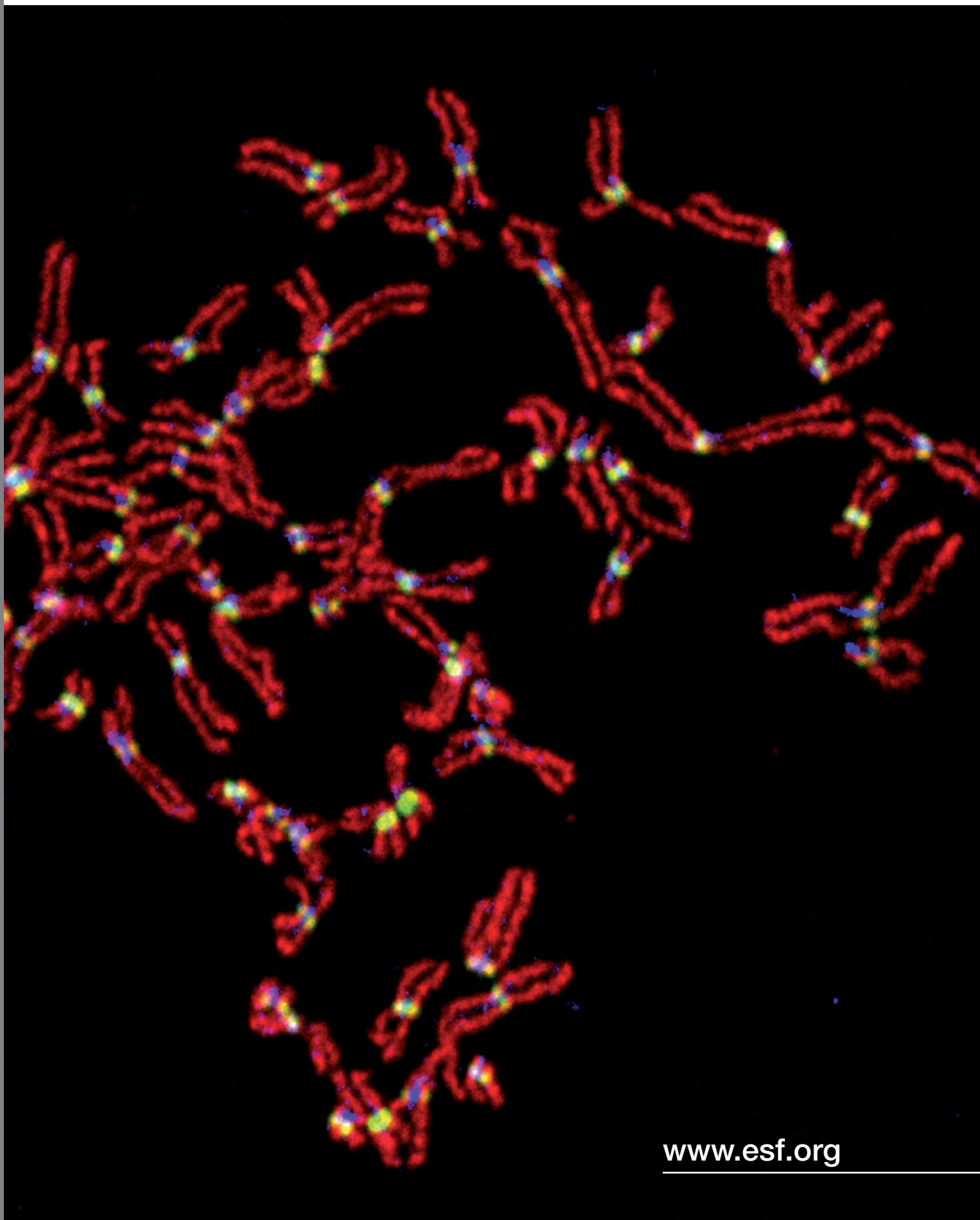
EUROPEAN SCIENCE FOUNDATION

SETTING SCIENCE AGENDAS FOR EUROPE

EUROCORES Programme

European Collaborative Research

EuroDYNA - Final Report



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General Information

What is EUROCORES?

The EUROCORES (European Collaborative Research) Scheme is a flexible framework that promotes excellence in collaborative research and networking. Offered by the European Science Foundation (ESF), EUROCORES tackles scientific questions in and across all disciplines by means of an integrated European or even global effort.

The Programmes encourage and foresee networking and collaboration of researchers to achieve synthesis of scientific results across the programme, to link to related programmes, and to disseminate results. EUROCORES Programmes allow national research funding organisations in Europe and beyond to support top class research in and across all scientific areas, by matching the needs articulated by the scientific community with their strategic priorities. Funding decisions on the projects and the research funding remain with the national research funding organisations, based on international peer review operated by ESF. ESF also provides support for networking the researchers and for the scientific synthesis of research results and their dissemination. Until December 2008 this is supported through a contract with the European Commission under the Sixth Framework Programme (EC Contract no. ERAS-CT-2003-980409). From January 2009 onwards this support will be provided by the national Funding Organisations participating in the Programmes. **www.esf.org/eurocores**

Programme Structure and Governing Bodies

A EUROCORES Programme is overseen by a *Management Committee* formed by one representative of each of the participating national funding agencies and the EUROCORES Programme Coordinator.

An international, independent *Review Panel* oversees the scientific aspects of the Programme. This includes assessment of outline proposals, selection of externally peer reviewed full proposals and the monitoring of the overall scientific progress of the Programme.

The *Scientific Committee* is formed by the Project Leaders of all funded Collaborative Research Projects (CRPs) and the EUROCORES Programme Coordinator. It is responsible for the networking and dissemination activities within the framework of the EUROCORES Programme.

Final Evaluation

Each EUROCORES Programme is subjected to a final evaluation by the Review Panel. The final evaluation concerns the overall achievements of the Programme as a whole and as such complements the evaluations of individual projects conducted at the national level. The merits of a Programme will be assessed on the basis of the scientific achievements highlighted by the Project Leaders as well as the usefulness and impact of the networking, training and dissemination activities undertaken. To this end, emphasis is placed on the activities which took place between the various CRPs with the aim to assess the added value of the Programme.

For the final evaluation of EuroDYNA, the Review Panel assessed the final reports of the CRPs and in addition attended the final EuroDYNA conference, during which the Project Leaders presented the highlights of their respective CRP. During a subsequent Review Panel meeting, the merits of the Programme and lessons to be learned were discussed.

This summary report is composed of three sections. The first one - EuroDYNA Recommendations - provides an overview of the Programme's achievements and recommendations for future research topics to scientists and funders; the second section highlights EuroDYNA's publications and in the last section, the Governing Bodies of EuroDYNA are presented.

Cover picture:
Spread of human mitotic chromosomes stained for the proteins condensin (red), cohesin (blue) and the centromere specific histone,CENPA (green). Micrograph by Peter Lenart, IMP, Vienna.

EUROPEAN SCIENCE FOUNDATION

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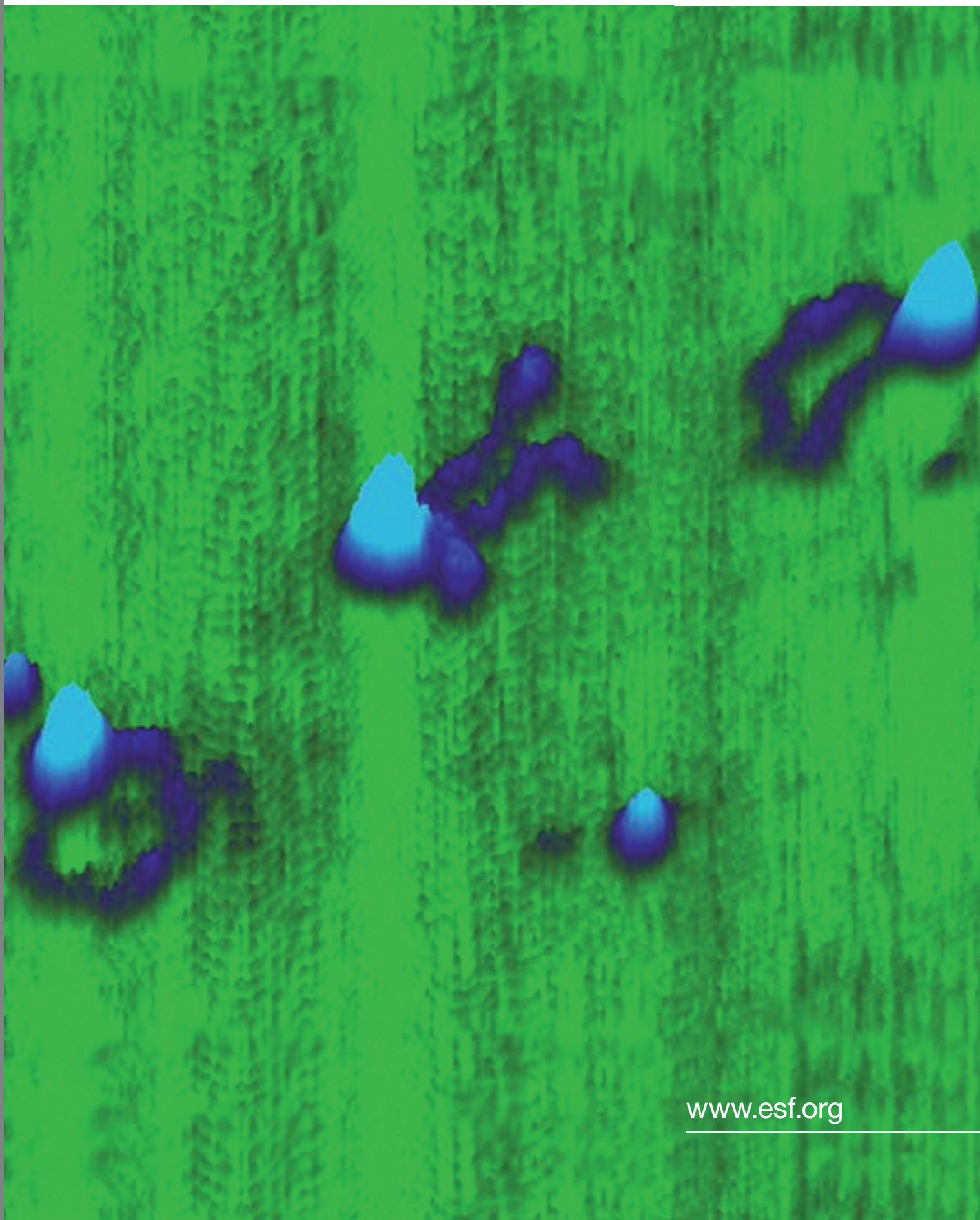
EUROCORES Programme
European Collaborative Research

Dynamic Nuclear Architecture and Chromatin Function (EuroDYNA)

EuroDYNA Recommendations



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Introduction

EuroDYNA[1], the European Collaborative Research (EUROCORES) Programme whose aim it was to shed light onto the functioning of the nucleus, the control centre of a cell, came to an end in 2008. Over a three-year period the Programme offered scientists the possibility of teaming up with peers and exploring new research directions in a flexible manner.

EuroDYNA accommodated nine thematic Collaborative Research Projects (CRPs), bringing together a total of 40 European research groups. Overall, the Programme combined expertise in different fields such as dynamic chromatin structure and nuclear architecture, regulation of gene expression, RNA processing and transport as well as genome surveillance. The latest technologies in molecular biology and biochemistry were employed together with advanced microscopy, structural analysis and computational approaches in order to gain a deeper insight into how the nucleus operates.

The Programme was funded by two sources: National funding agencies from eight European countries joined forces to provide a total of seven Million Euro to conduct research within the framework of EuroDYNA. This was complemented by a total of 170.000 Euro to support the networking and dissemination activities of funded scientists across CRPs, through the EU's Framework Programme 6.

The Programme's achievements

During its three-year life span, EuroDYNA offered its members a diverse array of networking opportunities, of which the annual conferences were particularly successful. This is illustrated by the fact that 17 new collaborations were formed between scientists across several thematic CRPs, a development that otherwise would not have happened. This is also where the added value of EuroDYNA kicked in as scientists with related yet slightly different research interests got together on a regular basis to present their data and have stimulating debates with the possibility of setting up new research initiatives. This interaction was further developed through short-term visits of students between the CRP labs.

EuroDYNA was also active beyond its boundaries, forging links with EU-networks and other EUROCORES Programmes within the same discipline and across scientific disciplines. For instance, in 2006 and 2007, two brain storming meetings took place involving members of EuroDYNA and SONS[2] (a EUROCORES Programme in the Physical Sciences) to facilitate cross-disciplinary exchange at the interface of molecular biology and material science/nanoscience. Within the Life Sciences, EuroDYNA members participated in a Mini-Symposium held by the EUROCORES RNAQuality in 2007 and the RNAQuality Training Workshop in 2008.

EuroDYNA held its last community event at The Wellcome Trust Conference Center in Hinxton, UK from 28-31 May 2008. This final conference highlighted the scientific achievements generated during the Programme's lifetime and provided a forum for discussion between EuroDYNA investigators and members of the EuroDYNA Review Panel. On the whole, EuroDYNA has produced numerous high-level publications, including articles in Nature and Cell, and the cross-CRP and cross-EUROCORES interactions have already successfully laid the foundation for joint publications and grant submissions.

Future prospects

EuroDYNA has yielded fruit and in order to build on the Programme's achievements, the EuroDYNA Review Panel and the EuroDYNA investigators discussed future opportunities in the field. Their recommendations, presented on the following pages, are meant to serve both the scientific community and funders alike; the scientific community for the development of new collaborative projects, the submission of a new EUROCORES theme proposal, etc.; the funders to raise awareness with regards to emerging topics to be supported on a national or transnational level across Europe.

Astrid Lunkes
EUROCORES Programme
Coordinator for EuroDYNA

[1] Dynamic Nuclear Architecture and Chromatin Function, [2] Self-Organised Nanostructures

EuroDYNA Review Panel - Consensus Report

The Programme is composed of nine Collaborative Research Projects (CRPs), with a fairly broad array of topics, focus and size. While the achievements vary between CRPs, the Panel was impressed by the overall output of papers produced during the Programme's 3-year lifetime. The integration of different disciplines was clearly considered an added value of the Programme as was the interaction between different CRPs. Most of these interactions were unforeseen and some have already led to joint CRP publications or grant submissions.

The Panel highlighted the added value of the networking activities, in particular the annual EuroDYNA conferences; these meetings proved to be an effective means to initiate new collaborations across CRPs and to generate new insights. The Panel was also very positive about the training possibilities the Programme offered to young scientists, be it within or across CRPs. They felt that it was important to expose the younger generation to the breadth and interdisciplinary character of the field.

The Panel indicated that the Programme did very well in terms of the dissemination of results to the scientific community at large. On the other hand, they felt that investigators should in general be more implicated in science communication with the lay public through open days/guided tours, newspaper interviews and articles, radio programmes etc..

On the whole, the Review Panel considered EuroDYNA a real success with all CRPs having been very productive in terms of publications, contributions to networking and training, as well as dissemination activities. While a lot of joint publications have already come out, more are to be expected in the future, especially those originating from recently started cross-CRP interactions.

The Panel recommended that EuroDYNA investigators build on the achievements of the Programme by:

1. Continuing to "create opportunities" through the organization of workshops/small conferences which could replace the annual EuroDYNA conference.

2. Linking up with other EUROCORES Programmes where possible (i.e. RNAQuality)

3. Submitting a new EUROCORES Theme proposal. A good concept for a Call today would be to focus on structural aspects, looking at different resolution ranges and encouraging the use of the latest technologies to address longstanding questions around the functioning of the nucleus.

Recommendations for Future Research Topics

When the EuroDYNA Call was published in 2003, the domain of nuclear dynamics was more difficult to study through lack of adequate equipment and technology. New developments and technological advances have since emerged and have opened new possibilities for future research in this domain.

Members of the EuroDYNA Review Panel and EuroDYNA investigators felt that a good concept for a Call in the near future would be to focus on structural aspects, looking at different resolution ranges and encouraging the use of the latest technologies to address longstanding questions around the functioning of the nucleus. Amongst others, investigation of the following subtopics could be envisaged:

- structure of higher order organisation of the nucleus and what controls it
- dynamics of nuclear structures and how these are controlled
- structural analysis of nuclear bodies
- regulation of nuclear processes in time
- three-dimensional control of gene expression: how gene/chromatin positioning affects expression capacity
- quantitative and theoretical approaches to analyse how molecules get together (molecular crowding)
- application of the use of new technologies to the study of chromatin conformation and function
- molecular mapping of gene contacts in the three dimensional nuclear space (using eg "chromosome conformation capture" (3C))
- organization of interphasic chromosomes
- intranuclear transport
- meiosis and oocyte maturation

  → could be developed into an own Call topic since it is highly relevant for research on fertility and genetic diseases.

Networking and Dissemination Activities

Annual Meetings

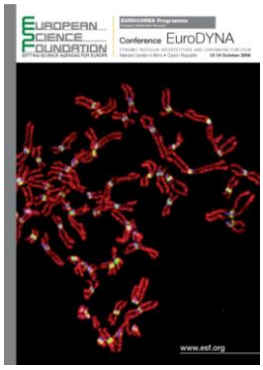
3rd EuroDYNA Meeting | 28 - 31 May 2008, The Wellcome Trust Conference Centre in Hinxton, UK

EuroDYNA takes lid off the genome
European researchers have made significant progress unravelling how genes are governed and why this sometimes goes wrong in disease. The key lies in the dynamic ever-changing structure of the chromatin, which...

EuroDYNA leaves healthy genomic research ecosystem as legacy
Europe's position as a major player in genome research has been boosted by the European Science Foundation's three-year EUROCORES Programme EuroDYNA. As it draws to a close, EuroDYNA is leaving behind a healthy European ecosystem of interacting...

read more at www.esf.org/eurodyna

2nd EuroDYNA Meeting | 12 - 14 October 2006, Gregor Mendel Center in Brno, CZ



EuroDYNA conference magnifies small components for big issues: finding the answer to human disease
At a recent EuroDYNA conference in Brno, Czech Republic, 60 scientists from nine European countries came together to present their research in the field of genetics and cell nucleus architecture...

Finding a cure for cancer: the holy grail of science
To find a cure for cancer, the modern-day plague of our society is synonymous to finding the holy grail of science...

read more at www.esf.org/eurodyna

Kick-off meeting | 22 - 24 September 2005, Thun, CH

The EuroDYNA community met for the first time to present their projects and to discuss the needs of the field and future activities; the importance of annual conferences was highlighted on this occasion.

Training

Fourth International Summer School on *DNA and Chromosomes* | 19 June - 1 July 2006, Corsica, FR

The Summer School aimed to integrate the various biological and physical approaches used to study DNA and chromosomes.

Short-term visits

EuroDYNA labs from different CRPs participated in the exchange of EuroDYNA students and postdocs.

Networking and Dissemination Activities

Topical Workshops

How we can benefit from each other

Impact of stress on the chromatin dynamics and global gene transcription in yeast and mammalian cells | 3 July 2006, Vienna, AU

Two of EuroDYNA's CRPs focusing on stress-induced global changes in gene expression in yeast and mammals respectively, came together to exchange techniques and reagents and to establish collaborations.

Establishing links with other European Projects

Chromatin-associated phosphorylation and dephorsphorylation | 18 - 20 January 2007, Vienna, AU

The workshop was dedicated to combine the systems biology experience of the EU-FP6 QUASI team with the experience of EuroDYNA groups for the development of novel approaches. This contributed to the submission of a joint article and joint grant proposal.

Activities across EUROCORES Programmes

Biologists meet physicists head on in 2006 and 2007

EuroDYNA – SONS brainstorming meetings | 4 December 2007, Lisbon, PT and 27 September 2006, Brussels, BE

ESF organised brainstorming meetings for investigators of the EUROCORES Programmes EuroDYNA and SONS (Self-organised Nanostructures) interested in, and working at the interface of molecular biology and physics. With biology becoming increasingly multidisciplinary ESF works to facilitate cross-disciplinary exchange. The meetings led to the introduction of short-term visits as networking activity for the EUROCORES Scheme at large and the submission of joint proposals.

RNAQuality establishes ties with EuroDYNA

Mini-Symposium on *RNA Biogenesis and Quality Control* | 18 September 2007, Aarhus, DK

The symposium was initiated to provide an efficient platform for establishing collaborations across CRPs within the RNAQuality Programme, as well as links to laboratories within the EuroDYNA Programme.

Workshop on *Structure and function of mRNP* | 4-8 August 2008, Aarhus, DK

This training workshop continued to foster links between RNAQuality and EuroDYNA. It involved PIs of both EUROCORES Programmes as lecturers. In addition, students from both the EuroDYNA and the RNAQuality network benefited from the event.

Networking and Dissemination Activities

Dissemination Events

International conference on *Telomeres and Genome Stability* | 30 August - 3 September 2006, Villars-sur-Ollon, CH

EuroDYNA was highlighted as sponsor of this international event. The visibility was further increased through the talk of EuroDYNA's Chair and poster presentations by EuroDYNA members.

Session on *Chromatin and Cell Cycle* at the ELSO meeting | 1 September 2007, Dresden, DE

Dissemination from the event:

- *At the ELSO meeting in Dresden in September 2007, members of the EuroDYNA community as well as invited speakers from the US and Canada came together for a EuroDYNA-organised session. On this occasion talks focused on the subject "Chromatin and the cell cycle" and the speakers covered everything from plant cells, via Drosophila cells to mammalian cells…*
- *Coling Logie, Chair of the EuroDYNA Scientific Committee, speaks about scientific achievements through the EUROCORES Programme EuroDYNA and his personal experience at the ELSO Conference, Dresden, Germany in September 2007…*

more at www.esf.org/eurodyna

A moment with Colin Logie

Colin Logie

A moment with Colin Logie

In a recent interview, Colin Logie, Chair of the EuroDYNA Scientific Committee, talks about organising the EuroDYNA session at ELSO and about future challenges for the cell biology field and for EuroDYNA.

Why did you choose the topic "Chromatin and the cell cycle" for the EuroDYNA session at ELSO?
Although we know a lot about the cell, DNA and chromatin, we still lack insight into how it functions. To understand how things function you have to put them into context. One thing about life is that it is cell based and one thing about cells is that they are always the product of the cell division of a previous cell. So, to really understand chromosomes we really have to understand how the chromosomes behave in the cell cycle. I think during the session we saw an example of very disparate talks ending up with conclusions about chromosomes which fitted together because they fit the context of the cell cycle as the common denominator.

What, in your opinion, are the challenges in your field?
One of the frontiers of this field is to really see what happens inside living cells. We have done many beautiful experiments in the recent past (by we, I mean the Scientific Community) but what we really need is multi-molecular assembly dynamics data. These things are very difficult to see at the moment and we really need to be able to see them to find out which factors are playing roles of messengers and which ones are playing more structural roles. Essentially it boils down to physically describing the isomerisations that take place in the cell, the DNA and also in the membranes. We also need to find ways of estimating the energetic code of each transaction and of integrating these types of data over multiple length scales from the nanometer to the micrometer. From this we should be able to formulate a mathematical description of biological systems.

What's also a big challenge is our ability to monitor things at the right timescale. We know that molecules function on the level of millions as well as thousands of a second and this spans six maybe even seven orders of magnitude. Right now we don't have good modelling systems to integrate all the data at those different time and length scales and I think that's a major challenge. It's not so complicated to address this. We need durable funding of scientific research; we need to maintain and sometimes also improve career opportunities, support institutes where innovation and originality are encouraged and promote communication amongst scientists. The latter is very important and something that ESF has been doing very well. We need communication between disciplines but also within disciplines.

What can the Scientific Community expect from EuroDYNA as a collaboration?
One field which is moving forward at the moment is nanoscience. By looking in great detail using biophysical methods on single molecules we are actually studying nanomotors which are driven by ATP. The exciting application for this is that maybe one day such motors can be harvested to produce DNA-based machines. EuroDYNA's contribution in this field involves what we are doing in defining the forces that are deployed by these motors. At the moment the physical description of biology is lagging behind but we are getting there now by finding forces, distances and time.

The full podcast with Colin Logie can be found on the EuroDYNA website at www.esf.org/eurodyna

The brains behind EuroDYNA

Niels Galjart

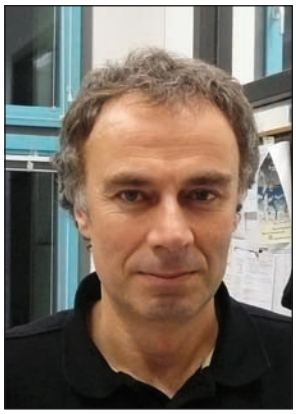
David Shore

EUROCORES is the European Science Foundation's flagship activity. It supports interdisciplinary research in non-traditional areas, thereby opening new horizons in science. With EuroDYNA, one of the EUROCORES Programmes, coming to an end, some of the Project Leaders have shared their experiences from the Programme with us.

"Thanks to EuroDYNA, nine research projects were funded that may otherwise not have been funded. Therefore, European research in the area of nuclear dynamics and architecture has been stimulated. Without EuroDYNA I would not have been able to perform the research I have carried out over the last three years. One aspect of EuroDYNA that I like a lot is the lack of bureaucratic burden compared to other research programmes. Another very important aspect is that the EUROCORES programmes are suggested by the scientists themselves (bottom-up approach). Finally, a great added value is the willingness at ESF to stimulate discussion among scientists, by organizing conferences, workshops and brainstorm meetings. As a EuroDYNA member I have benefited enormously from this valuable resource," said Niels Galjart, Department of Cell Biology and Genetics, Erasmus University, Rotterdam and the Project Leader of "Role of multi zinc finger proteins CTCF and BORIS in the dynamic change of the nuclear architecture and chromatin function during cell cycle and differentiation".

There is no doubt that EuroDYNA has achieved some great results and many of these results stem directly from the EUROCORES Programmes' focus on networking and collaboration. David Shore, University of Geneva and the Project Leader of "Environmental stress-induced dynamic modulation of chromatin structure, gene expression and nuclear architecture in yeast" commented, "My project recently entered into collaboration with a lab in Vienna. This wouldn't have happened if it weren't for EuroDYNA. The Vienna group is interested in understanding how arsenic affects cells and of course this has important global health implications. Arsenic is a pollutant in drinking water in many places in the world. Our collaboration began at a EuroDYNA meeting in Brno; we presented a gene we were working on that's involved in growth regulation in yeast cells and also in the cellular response to stress (which is what our project is aimed at understanding) when we were approached by a researcher from the Vienna group. The Vienna researcher noticed that this gene had also come up in his studies as a regulator of the cellular response to arsenic poisoning. As a result, we got together and did some more work which has now led to a manuscript ready for submission".

The brains behind EuroDYNA



Pavel Kovarik

René Ketting

EuroDYNA has been successful in generating new and exciting collaborations that have been hugely beneficial to the people involved. Now the scientists are focusing on what happens next. EuroDYNA is finishing but new collaborations have been set up.

"For me personally, the rather generous funding of the networking activities within EuroDYNA turned out to be very useful. Although I still maintain close links with the original members of my Collaborative Research Project (CRP), I have now made several links with members of other CRPs which are also relevant for my future research," said Pavel Kovarik, Vienna Biocenter Instititue of Microbiology and Genetics and the Project Leader of "Control of stress and interferon regulated gene expression by transcription factors, histone modification and nuclear compartmentalisation" during a recent interview.

This viewpoint is shared by René Ketting, Hubrecht Laboratory, Netherlands Institute for Developmental Biology and the Project Leader of "Nuclear actions of mRNAs". "I think scientific collaboration is essential. Many of my papers have resulted from collaborations that have been forged through meetings and exchange programmes. In my experience these collaborations are formed *de novo* on very different occasions, but a collective such as EuroDYNA is certainly a good catalyst for such interactions.

EuroDYNA is likely to have established new collaborations that will start to pay off in the future. I therefore think that the impact will not be limited to just the scientific progress that has been made during the funding period but will extend far beyond."

Find full profiles of these and other Project Leaders on the EuroDYNA website at www.esf.org/eurodyna

Cell biology of messenger RNA biogenesis

Abstract

Major events in the life cycle of a messenger RNA (mRNA) include transcription, splicing, 3' end processing, export from the nucleus to the cytoplasm, translation and degradation. These processes are intimately linked through proteins that bind to the mRNA in a specific and coordinated fashion. During the lifetime of an mRNA, the composition of associated protein complexes is under constant change. Through this Network the participating teams wish to study the dynamics of mRNA biogenesis making use of a wide range of multidisciplinary approaches. These include yeast genetics, molecular biology, structural biology, biochemical assays, proteomics, DNA microarrays, RNA interference, and live cell microscopy. The first Work Package of this proposal aims to dissect functional interactions between transcription initiation, RNA polymerase II, quality control and pre-mRNA processing events. The second Work Package is focused on the dynamics of pre-mRNA processing machines. The third Work Package aims to investigate the functional relevance of shuttling between the nucleus and the cytoplasm of proteins involved in mRNA biogenesis.

Partners

(FCT, FNU, SNF, NWO)

Prof Maria do Carmo-Fonseca (Project Leader)
University of Lisbon, Portugal

Dr Torben Heick Jensen
University of Aarhus, Denmark

Prof Walter Keller
University of Basel, Switzerland

Prof Jørgen Kjems
University of Aarhus, Denmark

Prof Angela Krämer-Bilbe
University of Geneva, Switzerland

Dr Ulrike Kutay
Swiss Federal Institute of Technology (ETH), Zurich, Switzerland

Dr Marc Timmers
University of Utrecht, The Netherlands

Role of multi zinc finger proteins CTCF and BORIS in the dynamic change of the nuclear architecture and chromatin function during cell cycle and differentiation

Abstract

The multi-zinc finger proteins BORIS and CTCF are unique and conserved factors with a role in transcriptional regulation, the organization of chromatin into distinct domains and imprinting. BORIS is expressed in the testis in cells that do not express CTCF. Abnormal upregulation of BORIS, on the other hand, may be linked to tumorigenesis. Thus, while binding to similar sites in the genome, these proteins could have distinct roles. We have generated inducible BORIS and CTCF knock out mice and are generating GFP- (or biotin)-tagged BORIS and CTCF knock in mice. From the inducible knock out mice cell lines have been isolated, which can be transfected with (mutant) multi-zinc finger proteins and/or DNA contructs with particular binding sites. Using these tools we will perform microscopic (live) imaging analysis (group Galjart), affinity purifications of biotin-tagged proteins (groups Galjart and Renkawitz) and structural analysis, like DNA loop formation (group Renkawitz) on the different types of mice, tissues and cells. This proposal aims at understanding the dynamic behaviour of both multi-zinc finger proteins during the cell cycle and the relevance of this behaviour and of these proteins for the maintenance of chromatin structure.

Partners

(DFG, NWO)

Dr Niels Galjart (Project Leader)
Erasmus University, Rotterdam, The Netherlands

Prof Rainer Renkawitz
Justus-Liebig-Universität, Giessen, Germany

Spatio-temporal organisation of genome surveillance in live cells

Abstract

Surveillance of the genome, which is vital for cellular function, cancer avoidance and many aspects of development, is comprised of a series of DNA repair and damage response pathways. Defects in damage surveillance result in severe genetic disorders. The mechanisms of these pathways are understood in varying degrees of detail, and the aim of this proposal is to understand the dynamics of the protein constituents within the cell nucleus before and after different DNA damaging treatments, as well as the inter-relationships between the different pathways. Normal and characterised mutant proteins tagged with GFP and its spectral variants, are either available from the participating laboratories or will be generated as part of the proposal. Motilities of the proteins are measured using variations of fluorescence recovery after photobleaching combined with whole cell or localised irradiation with either UV light or ionising radiation. The complementarity of the partners comes from their expertise in (1) different surveillance pathways and provision of tagged proteins; (2) advanced microscopic techniques; (3) delivery of different types of localised irradiation; (4) computer simulation. Through the integration of the different expertises, unique materials and reagents, and specialised equipment from the participating groups, the proposal forms a comprehensive and multidisciplinary approach to understanding the dynamics of genome surveillance in mammalian cells.

Partners

(DFG, FNU, MRC, NWO)

Dr Roland Kanaar (Project Leader)
Erasmus University, Rotterdam, The Netherlands

Prof Jiri Bartek
Institute of Cancer Biology, Copenhagen, Denmark

Prof Thomas Cremer
Ludwig-Maximilians Universität, Munich, Germany

Prof Günther Dollinger
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Dr Anna A. Friedl
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Dr Adriaan Houtsmuller
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Prof Alan Robert Lehmann
University of Sussex, UK

Dr Jiri Lukas
Institute of Cancer Biology, Copenhagen, Denmark

Prof Leon H.F. Mullenders
University of Leiden, The Netherlands

Dr Wim Vermeulen
Erasmus University, Rotterdam, The Netherlands

Nuclear action of miRNAs

Abstract

Double stranded RNA (dsRNA) is potent inducer of gene silencing. The mechanism by which these molecules induce silencing is evolutionary conserved, and represents a very powerful and specific way of gene activity control. One of the intermediates of this silencing process is a short RNA(Srna) molecule that has been named short interfering RNA (siRNA) or micro RNA (miRNA). These molecules act as guides for either an RNA degradation enzyme that is active in the cytosol or a complex that targets translation inhibition. In addition to these cytosolic events, nuclear effects of dsRNA have also been observed. In plants, dsRNA leads to methylation of homologous DNA sequences, and induces transcriptional silencing when promoter DNA is targeted. In yeast, dsRNA mediated processes have been implicated in centromere function. In animals, evidence for such nuclear effects has been obtained as well. For example, phenotypes of C. elegans mutants defective in RNAi suggest an impaired centromere function, and partially overlap with phenotypes associated with defects in the maintenance of silent chromatin states. In this research proposal we aim at a better understanding of the nuclear effects of dsRNA. We will do this by analyzing nuclear dsRNA processing, by identifying nuclear sRNA, by analyzing proteins associating with the nuclear sRNA and by analyzing the effects of nuclear sRNA on chromatin modifications and transcriptional activity.

Partners

(NWO, FWF)

Dr René F. Ketting (Project Leader)
Netherlands Institute for Developmental Biology, Utrecht, The Netherlands

Dr Majori Matzke
Austrian Academy of Sciences, Vienna, Austria

Control of stress and interferon regulated gene expression by transcription factors, histone modification and nuclear compartmentalisation

Abstract

The precise regulation of gene expression in response to extracellular stimuli plays a key role in life and biological diversity. Specific transcription factors, general transcription machinery, histone modifying enzymes, chromatin remodeling complexes, as well as nuclear architecture all have roles in gene transcription. How these individual events are coordinated in time and space, and integrated into appropriate transcriptional responses is a challenging yet unresolved question. We will address this issue using as a model the transcription of stress and interferon regulated genes. Both signalling pathways can be activated by independent stimuli and may therefore be studied separately. However, under physiological conditions, the stress and interferon signalling cascades are often activated simultaneously resulting in enhanced transcriptional responses. This synergism is essential for defense against pathogens and in tumor surveillance. Our studies on the order, location and contribution of stress and interferon-induced changes in chromatin modification and nuclear architecture will improve our understanding of the regulation of gene expression and open up new possibilities to combat diseases, such as cancer and infections. The availability of cells and animals with specific genes of the stress and interferon pathways inactivated will enable us to test the working hypotheses under physiological conditions.

Partners

(FWF, GAČR)

Dr Pavel Kovarik (Project Leader)
Institute of Microbiology and Genetics, Vienna, Austria

Dr Pavel Hozák
Institute of Molecular Genetics, Prague, Czech Republic

The role of linker histone variants and their phosphorylation in chromatin structure and function

Abstract

The linker histones are known to contribute to the formation and maintenance of higher order chromatin structures but their physiological functions are still largely unknown. They display a complex pattern of variants and recent data suggest that they may have specific roles in epigenetic control of gene expression. The cell cycle dependent phosphorylation of certain serine and threonine residues in the charged tails of the linker histones is most probably of major importance in determining the architecture of chromatin during cell proliferation and differentiation, but the molecular details of this process are very unclear. Aberrant chromatin structure may contribute to malignant transformation and tumour formation. This project aims at elucidating these mechanisms by combining the expertise of Herbert Lindner's laboratory in Innsbruck and Jean Thomas' laboratory in Cambridge, in collaboration with Ingemar Rundquist's laboratory in Linköping. All three research groups have many years' experience in linker histone research and the laboratories complement each other well, offering a large range of methods and techniques in analytical chemistry, structural biology, biophysics, and cytochemistry. Together, these investigations should contribute to increased understanding of epigenetic mechanisms involved in chromatin architecture, regulation of cell growth and differentiation, and in malignant transformation and tumour progression.

Partners

(FWF, MRC)

Prof Herbert Lindner (Project Leader)
University of Innsbruck, Austria

Prof Jean O. Thomas
University of Cambridge, UK

Associated Partner:
Prof Ingemar Rundquist
University of Linköping, Sweden

Chromatin higher order dynamics: a single molecule approach

Abstract

Higher order structure of eukaryotic chromosomes is governed by protein/DNA interactions that mediate the folding of DNA into chromatin fibres. Chromatin fibre structure revolves around nucleosomes, the fundamental units of chromatin. SNF2 ATPases and histone modifying enzymes remodel nucleosomes and have been documented to play key roles in the generation, maintenance and alteration of the epigenetic code during the cell cycle and during ontogeny. We propose to study the influence of chromatin remodelling factors on the physical properties of chromatin fibres. To this end, fully recombinant model polynucleosomal arrays suited for physico-chemical characterisation will be generated. Second, defined prototypic chromatin remodelling activities will be purified in preparative quantities. Last, state-of-the-art single molecule magnetic tweezers and time-lapse Atomic Force Microscopy will be employed to rigorously investigate the physical properties and dynamics of chromatin higher order structural transitions catalysed by chromatin remodellers. With this combined multidisciplinary approach we expect to elucidate nucleosome mediated higher order chromatin structural transitions with an unsurpassed degree of resolution.

Partners

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The control of chromosome structure by cohesion/ condensin complexes

Abstract

All organisms possess sophisticated mechanisms to respond to environmental stress. The robust transcriptional response to osmostress in yeast works through a conserved MAP kinase signaling pathway that leads to a major reprogramming of the cell's transcriptional state. The MAPK Hog1 acts directly at a large number of promoters to induce expression of osmostress-reponse genes, while a less well understood mechanism(s) causes coordinate down-regulation of growth-related genes, such as those encoding ribosomal proteins. Our primary aim will be to characterize and elucidate mechanisms underlying the dynamic changes in chromatin structure and modification states that accompany both the induction and down-regulation of genes following osmostress, using chromatin immunoprecipitation as our standard experimental tool. We will also employ high throughput genetic screens and advanced biochemical methods to understand how the chromatin remodeling / modification machinery interacts with global and gene-specific transcription factors and how these interactions promote dramatic changes in transcription rates. Finally, advanced cell biological methods (e.g. FRAP) will be used to characterize the dynamic nuclear trafficking and chromatin association of specific transcription factors following osmostress. These studies should yield new and important insights into mechanisms by which chromatin modification is linked to highly dynamic changes in transcription and overall nuclear architecture.

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Environmental stress-induced dynamic modulation of chromatin structure, gene expression and nuclear architecture in yeast

Abstract

All organisms possess sophisticated mechanisms to respond to environmental stress. The robust transcriptional response to osmostress in yeast works through a conserved MAP kinase signaling pathway that leads to a major reprogramming of the cell's transcriptional state. The MAPK Hog1 acts directly at a large number of promoters to induce expression of osmostress-reponse genes, while a less well understood mechanism(s) causes coordinate down-regulation of growth-related genes, such as those encoding ribosomal proteins. Our primary aim will be to characterize and elucidate mechanisms underlying the dynamic changes in chromatin structure and modification states that accompany both the induction and down-regulation of genes following osmostress, using chromatin immunoprecipitation as our standard experimental tool. We will also employ high throughput genetic screens and advanced biochemical methods to understand how the chromatin remodeling / modification machinery interacts with global and gene-specific transcription factors and how these interactions promote dramatic changes in transcription rates. Finally, advanced cell biological methods (e.g. FRAP) will be used to characterize the dynamic nuclear trafficking and chromatin association of specific transcription factors following osmostress. These studies should yield new and important insights into mechanisms by which chromatin modification is linked to highly dynamic changes in transcription and overall nuclear architecture.

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University of Pompeu Fabra, Barcelona, Spain

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Scanning (Atomic) Force miscroscopy image of the SMClike protein complex RAD50/MRE11/NBS1, involved in the early cellular response to DNA double-strand breaks. The complex consists of a globular DNA binding domain and two protruding coiled-coil arms (50 nm in length) that are required to tether broken DNA molecules.

We would like to thank the ESF Communications Unit for interviews and Jackie McLelland for editing and proofreading.

ANNEX

for ESF Member Organisations only

Cell biology of messenger RNA biogenesis

Abstract

Major events in the life cycle of a messenger RNA (Mrna) include transcription, splicing, 3' end processing, export from the nucleus to the cytoplasm, translation and degradation. These processes are intimately linked through proteins that bind to the mRNA in a specific and coordinated fashion. During the lifetime of an mRNA, the composition of associated protein complexes is under constant change. Through this Network the participating teams wish to study the dynamics of mRNA biogenesis making use of a wide range of multidisciplinary approaches. These include yeast genetics, molecular biology, structural biology, biochemical assays, proteomics, DNA microarrays, RNA interference, and live cell microscopy. The first Work Package of this proposal aims to dissect functional interactions between transcription initiation, RNA polymerase II, quality control and pre-mRNA processing events. The second Work Package is focused on the dynamics of pre-mRNA processing machines. The third Work Package aims to investigate the functional relevance of shuttling between the nucleus and the cytoplasm of proteins involved in mRNA biogenesis.

Partners

(FCT, FNU, SNF, NWO)

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Dr Torben Heick Jensen
University of Aarhus, Denmark

Prof Walter Keller
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Part A. Collaborative Research Project (CRP)

A.1. General information about the Collaborative Research Project (CRP)

CRP Number: 03-DYNA-F-03
CRP Title and Acronym: **Cell biology of messenger RNA biogenesis/ mRNA biogenesis**
Name Project Leader: Professor Maria do Carmo-Fonseca
Institutional website: www.imm.ul.pt
Project-related website: n.a.
Reporting period: from 01/01/2005 to 01/01/2008 (Please report for the whole duration of your project)

List all Principal Investigators (PIs), Associated Partners (APs) and Co-operating Partners (CPs) of the CRP

PI 1
name: **Walter Keller**
PI 2
name: **Angela Krämer**
PI 3
name: **Ulrike Kutay**
PI 4
name: **Torben Heick Jensen**
PI 5
name: **Jørgen Kjems**
PI 6
name: **H.Th. Marc Timmers**
PI 7
name: **Maria Carmo-Fonseca**

A.3. Report of the CRP in terms of Networking, Training and Dissemination

→ (To be completed by Project Leader with input from CRP members)

Please provide a concise and clear description of the networking, training and dissemination activities undertaken by this CRP, addressing the following issues:

6. Networking activities: Of all the networking activities developed during this period, please highlight the most important one for this CRP (in terms of outcome, impact, opportunities for trans-national collaborations and synergy with other European and international initiatives)

This CRP organized 3 Annual Meetings that were held in Lisboa, August 18-19, 2005; Aarhus, December 13-14, 2006 and Zurich, January 10-11, 2008. Each meeting was attended by most PIs as well as post-docs and PhD students from each team. These meetings provided an excellent forum for informal discussion of work in progress. New collaborative projects and other initiatives have originated during these meetings.

7. Training activities: Please, highlight the most useful training activity (workshop, course or school) undertaken by senior or junior researchers of this CRP

All activities represented excellent training opportunities that attracted researchers from different backgrounds pending on personal scientific interests.

8. Dissemination activities: What has this CRP done to promote the EuroDYNA Programme more actively and widely? Please, indicate the outcome and impact of the dissemination activities developed during this period

The scientific results obtained have been published in international peer reviewed journals and presented at international Conferences.

Three members of this CRP (Krämer, Kjems and Carmo-Fonseca) take part in the European Network of Excellence on Alternative Splicing (EURASNET).

All participating Principal Investigators were regularly invited to present the outcome of the scientific activities supported by EuroDYNA at international meetings.

All participating Principal Investigators lead international teams and provide training to PhD students and junior researchers from across Europe.

A.4 Valuable feedback from this CRP

→ (To be completed by Project Leader with input from CRP members)

Please, provide a <u>concise and clear answer</u> to these questions:

9. Key contribution of this CRP to the EURODYNA Programme: How has the EuroDYNA Programme benefited from this CRP? Please, provide one example that clearly illustrates your valuable input (whether scientific, networking, training and/or dissemination input)

Understanding how information contained in the genes is “decoded” into molecules represents a key challenge to know how genomes function. This CRP combines biologists, biochemists and geneticists to tackle the problem of mRNA biogenesis in the context of control of gene expression. Working on a variety of model organisms (yeast, virus, mammals) and using a wide range of methodologies (live cell microscopy, proteomics, RNAi, in vitro assays) the scientists in this CRP are contributing fundamental novel knowledge to the fields of transcription and mRNA processing.messenger RNA

10. Key contribution of the EURODYNA Programme to this CRP: How has your participation in the EuroDYNA Programme influenced your research (in terms of visibility, collaborations, opportunities, ideas)

The EuroDYNA Conferences represented an important forum for networking with other CRPs. Collaborators from other CRPs include Houtsmuller (FRAP experiments) and Jan Ellenberg (live cell microscopy).

A.5 Follow up related to the CRP and EuroDYNA Programme

→ (To be completed by Project Leader with input from CRP members)

11. Follow up: Please provided details of the most important new initiatives (either within a national or an international context) that have been developed as a result of the collaboration of this CRP and the EuroDYNA Programme.

A member of this CRP (T. H. Jensen) has initiated collaboration with a new ESF Network on RNA quality (NuRNASu).

Members of this CRP are preparing applications to collaborative research grants under European Commission FP7.

A. 5. List of Publications, Dissemination and Outreach Activities undertaken by the CRP

→ (To be completed by Project Leader with input from CRP members)

Publications

- Articles

Peer reviewed articles in journals (published, in press or submitted)

***** Kyburz, A., A. Friedlein, H. Langen, and W. Keller: Direct interactions between subunits of CPSF and the U2 snRNP contribute to the coupling of pre-mRNA 3' end processing and splicing. Mol. Cell 23, 195-205 (2006).

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Published contributions to international conferences

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Other articles (*please define*)

- Books

As editor(s)

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Carmo-Fonseca, M. and Carvalho, C. (2007). Nuclear Organization and Splicing Control. In "Alternative Splicing in the Postgenomic Era", ed. B.J. Blencowe and B. R. Graveley, Landes Bioscience.

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Lutzelberger M, Kjems J.Strategies to identify potential therapeutic target sites in RNA. Handb Exp Pharmacol. 2006;(173):243-59.

- Other (*please define*; *e.g.*, data products, videos)

Presentations in Scientific Meetings

- Oral presentations (indicate invited / keynote talks)

Walter Keller: “A new yeast poly(A) polymerase complex involved in RNA quality control”

EMBO Workshop Messenger RNA 3’ ends: Interconnections with transcription and mRNA turnover, Brasenose College, University of Oxford UK, March 2005 (invited talk).

Monika Garas: “Potential roles of yeast CPF subunits Ysh1p and Syc1p in pre mRNA 3’ end processing and RNA polymerase II transcription termination“

European RNOMICS Network Meeting, University of Lisbon, Portugal. March 2005 (invited talk).

Andrea Kyburz: “CPSF and U2 snRNP mediate coupling of pre-mRNA 3’ end processing and splicing“ Eukaryotic mRNA Processing, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA, August 2005 (invited talk).

Walter Keller: “Polyadenylation-mediated quality control of RNA”

ZMBH Forum “Cellular Quality Control”, Center for Molecular Biology, University of Heidelberg, September 2006 (invited talk).

Angela Krämer: *Analysis of SF1 function in alternative splicing*. EMBO Conference Series “1st Meeting on Pre-mRNA processing and disease”, Cortina d’Ampezzo, Italy, 14-17 January 2007 (invited talk).

Angela Krämer was co-organizer of the SKMB-Swiss RNA Workshop in Bern, Switzerland, 14 September 2007

Ulrike Kutay: Research Seminar, Medizinische Fakultät, Tübingen, Germany. 2005 (invited talk)

Ulrike Kutay: Seminar for the Department of Biology, ETHZ, Switzerland 2005 (invited talk)

Ulrike Kutay: Talk at Minisymosium "Cellular Biochemistry", Gene Center Munich, Switzerland, 2005 (invited talk)

Ulrike Kutay: Special Research Seminar at Institute of Biochemistry, ETHZ, Switzerland 2005 (invited talk)

Ulrike Kutay: Cold Spring Harbor Meeting, August 2005, Invited Session chair, USA

Ulrike Kutay: EMBO/ FEBS Conference on Nuclear Structure and Dynamics, La Grande Motte, France 2005 (invited talk)

Ulrike Kutay: 2005 Biennial Nucleocytoplasmic Transport Meeting: Molecules, Mechanisms, and New Frontiers, Jekyll Island, October 2005, USA, Invited Speaker

Ulrike Kutay: Symposium 'Young talents in bioscience and biotechnology', February 2006, ETH, Switzerland

Ulrike Kutay: Research Seminar, Medizinische Fakultät, June 2006, Göttingen, Germany

Ulrike Kutay: Bioscience 2006: bioscience for the 21st century, July 2006, Glasgow, Great Britain

Ulrike Kutay: Gesellschaft für Virologie, Workshop 'Cell biology of viral infections', September 2006, Deidesheim, Germany, Invited Speaker

Ulrike Kutay: Symposium DFG-Graduiertenkollegs 745 - Mukosale Wirts -Erreger Interaktionen, December 2006, Hannover, Germany, Invited Speaker

Ulrike Kutay: Gordon research conference 'Signal transduction within the nucleus', March 2007, Ventura, CA, USA, Invited Speaker, Session Chair

Ulrike Kutay: Seminar at the Max-Delbrück Center for Molecular Medicine, Berlin, Germany

Ulrike Kutay: Minisymposium DKFZ Heidelberg, Germany 2007

Ulrike Kutay: Special Research Seminar at Institute of Biochemistry, ETHZ, Switzerland 2007

Ulrike Kutay: RNA 2007, Madison, USA, Invited Session Chair

Ulrike Kutay: FEBS 2007, Vienna, Invited Speaker

Ulrike Kutay: 63rd Harden conference, 2007 Invited Speaker

Ulrike Kutay: The biannual nuclear transport meeting, Sicily, Italy, 2007 Invited Speaker

T. H. Jensen was invited speaker and symposium organizer at ELSO 2005, Dresden, Germany

T. H. Jensen organized the meeting NorFA 2005, RNA biology, AU, Denmark

T. H. Jensen was invited speaker and chair in session on “Gene expression” EMBO Young Investigator program meeting, Vienna, Austria

T. H. Jensen was invited speaker at FASEB meeting on *”Posttranscriptional regulation of gene expression”*, Snowmass, Colorado, USA

T. H. Jensen was invited speaker at TERM 2006, Gif sur Yvette, Paris.

T. H. Jensen was invited speaker at EuroDYNA meeting, BRNO, Czech Republic, Oct. 12-15, *“Severely compromised transcription due to a 5’ splice site mutation”*.

T. H. Jensen was invited speaker at EURASNET meeting Barcelona, Spain, April 22-24, *“Interplay between transcription, splicing and degradation”*.

H.Th. Marc Timmers: “Regulation of transcription by RNA polymerase II by the multi-functional Ccr4-Not complex” at the University of Aarhus, Denmark. February 17, 2005 (invited talk)

H.Th. Marc Timmers: “Interplay between SAGA, Taf1p, Mot1p and chromatin remodelling in rapid promoter induction pathways” at the MCRI Oxted, Transcription workshop, UK May 14-15, 2005 (invited talk)

H.Th. Marc Timmers: "Regulation of transcription by RNA polymerase II by the multi-functional Ccr4-Not complex” at the 30th FEBS Congress & 9th IUBMB Conference Budapest, Hungary. July 4, 2005 (invited talk)

H.Th. Marc Timmers: “Regulation of Core Promoters” at the Biological Research Center of Hungarian Academy of Sciences, Szeged, Hungary. October 28, 2005 (invited talk)

H.Th. Marc Timmers: “Mobility of TBP and TBP complexes in Human cells” at the Cornell University in Ithaca, USA. April 20, 2006 (invited talk)

H.Th. Marc Timmers: “Mobility of TBP and TBP complexes in Human cells” at the Keystone Symposium, Regulation of eukaryotic transcription: from chromatin to mRNA, Taos New Mexico, USA. April 23, 2006 (invited talk)

H.Th. Marc Timmers: “Mobility of TBP and TBP complexes”, at the EMBL Transcription Meeting Heidelberg, Germany. August 26-30, 2006 (invited talk)

H.Th. Marc Timmers: “Mobility of TBP and TBP-complexes” at the Autumn symposium NVBMB, Rotterdam, The Netherlands. September 29, 2006 (invited talk)

H.Th. Marc Timmers: “Dynamic regulation of transcription by RNA polymerase II”, at the symposium ”Hot topics in Eucaryotic Transcription”, Jagiellonian University, Krakow, Poland October 2-4, 2006 (invited talk)

H.Th. Marc Timmers: “TFIID: histone methylation and mitosis” at the CRG, Barcelona, Spain. September 27, 2007 (invited talk)

H.Th. Marc Timmers: “Regulation of activity and distribution of the TATA-binding protein” at the workshop “Mechanistic and integrative aspects or mRNA synthesis”, UNIA, Baeza, Spain. October 2, 2007 (invited talk)

H.Th. Marc Timmers: “TFIID: histone methylation and mitosis” on the 4th Abcam Chromatin meeting, Antigua. November 30, 2007 (invited talk)

H.Th. Marc Timmers: “TFIID: histone methylation and mitosis”, IGBMC, Strassbourg, France. December 14, 2007 (invited talk)

Gama-Carvalho, M. *Genomewide identification of mRNA molecules associated with the nucleocytoplasmic shuttling splicing factors U2AF^{65} and PTB* (invited talk).

Dynamic Nuclear Architecture and Chromatin Function - First EuroDYNA Conference. Thun, Switzerland. 22 - 24 September 2005.

Gama-Carvalho, M. et al *The polypirimidine-binding splicing factors U2AF65 and PTB associate with functionally distinct subsets of mRNA molecules post-splicing* (Platform presentation). Cold Spring Harbor Laboratory Meeting on Eukaryotic mRNA processing. Cold Spring Harbor, New York. 24-28 August 2005.

Carmo-Fonseca, M. *Organization of nuclear proteins*. Plenary Lecture at the 30th FEBS Congress, Budapest, Hungary, 7 July 2005.

Carmo-Fonseca, M. *Dynamic organization of spliceosome components in the human cell nucleus*. EMBO/FEBS Conference on Nuclear Structure and Dynamics, La Grande Motte, France, 24-28 September 2005 (invited talk).

Carmo-Fonseca, M “Connections between pre-mRNA splicing, Pol II transcription and mRNA release” Cold Spring Harbor Laboratory Meeting on “Dynamic Organization and Nuclear Function”, September 2006 (invited talk)

Carmo-Fonseca, M “Coupling pre-mRNA processing to release from the site of transcription” at the EuroDyna conference, Brno, Czech Republic. October 12-14, 2006 (invited talk)

Carmo-Fonseca, M. *Tissue specific differences in splicing factor expression levels*. EMBO Conference “pre-mRNA processing and disease” Cortina d’Ampezo, Italy, January 14-17, 2007. (invited talk)

Carmo-Fonseca, M. *Intranuclear trafficking of mRNA and spliceosomal components: a stochastic view*. EMBO Workshop “Intracellular RNA localization and localized translation” Il Ciocco, Italy, July 1-6, 2007. (invited talk)

Carmo-Fonseca, M. *A stochastic view of mRNA biogenesis*. Instituto de Parasitologia y Biomedicina López Neyra, Granada, Spain, September 28, 2007. (invited talk)

Carmo-Fonseca, M. co-organized the Twelfth Annual Meeting of the RNA Society. University of Wisconsin-Madison, USA. May 29-June 3, 2007.

Carmo-Fonseca, M. chaired the Plenary Session on RNA at ELSO 2007. Dresden Germany, September 1-4, 2007.

Gama-Carvalho, M. co-organized the 4th National RNA Biology Meeting held at Vimeiro, Portugal. November 8-10, 2007.

- Posters

Monika Garas, Bernhard Dichtl and Walter Keller : « The role of Ysh1p/Brr5p in transcription termination and in 3' end processing of RNA polymerase II transcripts ». Swiss RNA Workshop, University of Bern, October 13, 2006.

Choleza, M., Tanackovic, G., Krämer, A. Human splicing factor SF1 localizes to parapeckles. EMBO Conference Series "Nuclear structure and dynamics", Montpellier, France, 1-5 September 2007.

Choleza, M., Tanackovic, G., Krämer, A. Human splicing factor SF1 localizes to parapeckles. SKMB Swiss RNA Workshop, Bern, Switzerland, 14 September 2007.

Kozlova, N. "AG-dependent and AG-independent variants of IgM form distinct commitment complexes." Poster presented at the EMBO Meeting on pre-mRNA processing and disease held in Cortina d´Ampezzo, BL, Italy, January 14-17, 2007.

Braga, J. "A reaction-diffusion model to study RNA motion by quantitative fluorescence recovery after photobleaching" Poster presented at 'Computational Cell Biology' Cold Spring Harbor Laboratory, New York, USA, March 6 -9, 2007.

Gama-Carvalho, M. "Post-transcriptional control of gene expression by the mammalian splicing factors U2AF^{65} and PTB" Poster presented at 'Systems Biology – Global regulation of gene expression' Cold Spring Harbor Laboratory, New York, USA, March 29 – April 2, 2007.

Public Outreach:

- Press releases

M. Carmo-Fonseca prepared a Press release describing the highlights of the CRP to the Portuguese News Agency

T. H. Jensen prepared Press releases on the establishment of the Centre for mRNP Biogenesis and Metabolism.

T. H. Jensen prepared a Press release on the publication by Damgaard et al Mol. Cell 2008. The story was brought in the Danish newspaper "Politiken" as well as on a scientific web page driven by the French embassy in Denmark.

- National / international Newspaper articles (presenting your CRP or part of your work)

An interview with M. Carmo-Fonseca presenting the CRP was published in one of the main Portuguese Newspapers (Publico)

Søren Lykke-Andersen and T. H. Jensen authored an article entitled *"Cellens budbringere"* Weekend avisen (tillæget Ideer). The article describes the concept of mRNPs and describes ways in which they serve to increase genome plasticity.

- TV appearance

M. Carmo-Fonseca participated in a talk show (Portuguese national television)

J. Kjems appeared in an Educational program on RNAi (Danish national television)

J. Kjems appeared in News on siRNA delivery (Danish national television)

- Radio appearance

M. Carmo-Fonseca was interviewed for a Radio program

J. Kjems appeared in Feature program on peronalized medicin

J. Kjems appeared in Feature program on viral evolution and treatment

Other Activities / Products

- Patents

Kjems, J. and Wengel, J: Small internally segmented interfering RNA (2006)

Howard, K., Xiu, X., Besenbacher, F., Kjems, J.: Nanoparticles for nucleic acid delivery (2006)

Andersen, M.O., Howard, K., Besenbacher, F., Kjems, J.: Dehydrated chitosan nanoparticles (2007)

Andreasen, M, Howard, K., Besenbacher, F., Kjems, J.: Osteopontin nanoparticle system for drug delivery (2007)

Howard, K., Besenbacher, F., Kjems, J.: Nanoparticle-mediated treatment for inflammatory diseases (2007)

IMPORTANT: In your list, please identify with an asterisk (*) those publications and contributions in which the EuroDYNA Programme is properly acknowledged.

Role of multi zinc finger proteins CTCF and BORIS in the dynamic change of the nuclear architecture and chromatin function during cell cycle and differentiation

Abstract

The multi-zinc finger proteins BORIS and CTCF are unique and conserved factors with a role in transcriptional regulation, the organization of chromatin into distinct domains and imprinting. BORIS is expressed in the testis in cells that do not express CTCF. Abnormal upregulation of BORIS, on the other hand, may be linked to tumorigenesis. Thus, while binding to similar sites in the genome, these proteins could have distinct roles. We have generated inducible BORIS and CTCF knock out mice and are generating GFP- (or biotin)-tagged BORIS and CTCF knock in mice. From the inducible knock out mice cell lines have been isolated, which can be transfected with (mutant) multi-zinc finger proteins and/or DNA contructs with particular binding sites. Using these tools we will perform microscopic (live) imaging analysis (group Galjart), affinity purifications of biotin-tagged proteins (groups Galjart and Renkawitz) and structural analysis, like DNA loop formation (group Renkawitz) on the different types of mice, tissues and cells. This proposal aims at understanding the dynamic behaviour of both multi-zinc finger proteins during the cell cycle and the relevance of this behaviour and of these proteins for the maintenance of chromatin structure.

Partners

(DFG, NWO)

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Prof Rainer Renkawitz
Justus-Liebig-Universität, Giessen, Germany

Part A. Collaborative Research Project (CRP)

→ (To be completed by Project Leader)

A.1. General information about the Collaborative Research Project (CRP)

CRP Number: 03-DYNA-F-10
CRP Title and Acronym: **Role of multi zinc finger proteins in the dynamic change of the nuclear architecture during cell cycle and differentiation/ multi-zinc finger proteins and the dynamic nuclear architecture**
Name Project Leader: Niels Galjart
Project-related website: http://www.univie.ac.at/eurodyna/
Reporting period: from 01/11/2004 to 01/11 /2007 (Please report for the whole duration of your project)

List all Principal Investigators (PIs), Associated Partners (APs) and Co-operating Partners (CPs) of the CRP

PI 1
name: **Niels Galjart**
PI 2
name: **Rainer Renkawitz**

A.3. Report of the CRP in terms of Networking, Training and Dissemination

→ (To be completed by Project Leader with input from CRP members)

Please provide a <u>concise and clear</u> description of the networking, training and dissemination activities undertaken by this CRP, addressing the following issues:

6. Networking activities: Of all the networking activities developed during this period, please highlight the most important one for this CRP (in terms of outcome, impact, opportunities for trans-national collaborations and synergy with other European and international initiatives)

see point A.2.4: a collaboration between this CRP and 03-DYNA-F-29.

7. Training activities: Please, highlight the most useful training activity (workshop, course or school) undertaken by senior or junior researchers of this CRP

Two types of activity were appreciated very much: the EuroDYNA conferences and exchange visits between Rotterdam and Giessen (the latter were funded by other means).

8. Dissemination activities: What has this CRP done to promote the EuroDYNA Programme more actively and widely? Please, indicate the outcome and impact of the dissemination activities developed during this period

Dissemination was done verbally, at meetings (for example the ELSO 2007 in Dresden) and in seminars. We have advertized how positive and beneficial the structure of ESF-based programs is for science. However, one should bear in mind that EuroDYNA is relatively small and it does not “run” for a long period of time, hence its impact can not be very big.

A.4 Valuable feedback from this CRP

→ (To be completed by Project Leader with input from CRP members)

Please, provide a <u>concise and clear answer</u> to these questions:

9. Key contribution of this CRP to the EURODYNA Programme: How has the EuroDYNA Programme benefited from this CRP? Please, provide one example that clearly illustrates your valuable input (whether scientific, networking, training and/or dissemination input)

I would like to refer to the interim report of ~1 year ago. This CRP is the smallest in the EuroDYNA program and its contribution is therefore limited. We have participated in shaping EuroDYNA and its contacts with other EUROCORES themes. PI1 of this CRP is co-organizing the last EuroDYNA conference in Hinxton, Cambridge (UK).

10. Key contribution of the EURODYNA Programme to this CRP: How has your participation in the EuroDYNA Programme influenced your research (in terms of visibility, collaborations, opportunities, ideas)

There was a good mix of different disciplines and research subjects, certainly for such a small program. This almost automatically generated ideas, both in areas of research that are not covered by our CRP and in the areas that are covered.

A.5 Follow up related to the CRP and EuroDYNA Programme

→ (To be completed by Project Leader with input from CRP members)

11. Follow up: Please provided details of the most important new initiatives (either within a national or an international context) that have been developed as a result of the collaboration of this CRP and the EuroDYNA Programme.

A member of another CRP is currently applying for an independent group leader position in Rotterdam. Together with universities from Giessen and Marburg the Erasmus MC is preparing an international grant application in the field of chromatin.

A. 5. List of Publications, Dissemination and Outreach Activities undertaken by the CRP

→ (To be completed by Project Leader with input from CRP members)

Publications

- Articles

Peer reviewed articles in journals (published, in press or submitted)

Joint publications:

Burke, L.J., Zhang, R., Bartkuhn, M., Tiwari, V.K., Tavoosidana, G., Kurukuti, S., Weth, C., Leers, J., Galjart, N., Ohlsson, R., and Renkawitz, R. (2005). CTCF binding and higher order chromatin structure of the H19 locus are maintained in mitotic chromatin. Embo J 24, 3291-3300.

Wesoly, J., Agarwal, S., Sigurdsson, S., Bussen, W., Van Komen, S., Qin, J., van Steeg, H., van Benthem, J., Wassenaar, E., Baarends, W.M., Ghazvini, M., Tafel, A.A., Heath, H., Galjart, N., Essers, J., Grootegoed, J.A., Arnheim, N., Bezzubova, O., Buerstedde, J.M., Sung, P., and Kanaar, R. (2006). Differential contributions of mammalian Rad54 paralogs to recombination, DNA damage repair, and meiosis. Molecular and cellular biology 26, 976-989.

Joint **submitted** work:

Van de Nobelen, S., Leers, J., Rosa-Garrido, M., Heath, H., Akhmanova, A., Torrano, V., Grosveld, F., Delgado, M.D., Renkawitz, R., Sleutels, F., and Galjart, N. (2008). CTCF defines the local epigenetic state of the ribosomal DNA repeat.

Heath, H., Ribeiro de Almeida, C., Dingjan, G., Van de Nobelen, S., Sleutels, F., Jonkers, I., Renkawitz, R., Grosveld, F., Hendriks, R.W., and Galjart, N. (2008). An essential role for CTCF in proliferation and differentiation during β-selection in the thymus.

Separate publications:

Mohan, M., Bartkuhn, M., Herold, M., Philippen, A., Heinl, N., Bardenhagen, I., Leers, J., White, R.A., Renkawitz-Pohl, R., Saumweber, H., and Renkawitz, R. (2007). The Drosophila insulator proteins CTCF and CP190 link enhancer blocking to body patterning. The EMBO journal 26, 4203-4214.

Holohan, E.E., Kwong, C., Adryan, B., Bartkuhn, M., Herold, M., Renkawitz, R., Russell, S., and White, R. (2007). CTCF Genomic Binding Sites in Drosophila and the Organisation of the Bithorax Complex. PLoS genetics 3, e112.

Rathke, C., Baarends, W.M., Jayaramaiah-Raja, S., Bartkuhn, M., Renkawitz, R., and Renkawitz-Pohl, R. (2007). Transition from a nucleosome-based to a protamine-based chromatin configuration during spermiogenesis in Drosophila. Journal of cell science 120, 1689-1700.

Splinter, E., Heath, H., Kooren, J., Palstra, R.J., Klous, P., Grosveld, F., Galjart, N., and de Laat, W. (2006). CTCF mediates long-range chromatin looping and local histone modification in the beta-globin locus. Genes & development 20, 2349-2354.

IMPORTANT: In your list, please identify with an asterisk (*) those publications and contributions in which the EuroDYNA Programme is properly acknowledged.

Spatio-temporal organisation of genome surveillance in live cells

Abstract

Surveillance of the genome, which is vital for cellular function, cancer avoidance and many aspects of development, is comprised of a series of DNA repair and damage response pathways. Defects in damage surveillance result in severe genetic disorders. The mechanisms of these pathways are understood in varying degrees of detail, and the aim of this proposal is to understand the dynamics of the protein constituents within the cell nucleus before and after different DNA damaging treatments, as well as the inter-relationships between the different pathways. Normal and characterised mutant proteins tagged with GFP and its spectral variants, are either available from the participating laboratories or will be generated as part of the proposal. Motilities of the proteins are measured using variations of fluorescence recovery after photobleaching combined with whole cell or localised irradiation with either UV light or ionising radiation. The complementarity of the partners comes from their expertise in (1) different surveillance pathways and provision of tagged proteins; (2) advanced microscopic techniques; (3) delivery of different types of localised irradiation; (4) computer simulation. Through the integration of the different expertises, unique materials and reagents, and specialised equipment from the participating groups, the proposal forms a comprehensive and multidisciplinary approach to understanding the dynamics of genome surveillance in mammalian cells.

Partners

(DFG, FNU, MRC, NWO)

Dr Roland Kanaar (Project Leader)
Erasmus University, Rotterdam, The Netherlands

Prof Jiri Bartek
Institute of Cancer Biology, Copenhagen, Denmark

Prof Thomas Cremer
Ludwig-Maximilians Universität, Munich, Germany

Prof Günther Dollinger
Technische Universität Munich, Germany

Dr Anna A. Friedl
Ludwig-Maximilians Universität, Munich, Germany

Prof Jan H.J. Hoeijmakers
Erasmus University, Rotterdam, The Netherlands

Dr Adriaan Houtsmuller
Erasmus University, Rotterdam, The Netherlands

Prof Alan Robert Lehmann
University of Sussex, UK

Dr Jiri Lukas
Institute of Cancer Biology, Copenhagen, Denmark

Prof Leon H.F. Mullenders
University of Leiden, The Netherlands

Dr Wim Vermeulen
Erasmus University, Rotterdam, The Netherlands

Part A. Collaborative Research Project (CRP)

→ (To be completed by Project Leader)

A.1. General information about the Collaborative Research Project (CRP)

CRP Number: 03-DYNA–F-18

CRP Title and Acronym: **Spatio-Temporal Organisation of Genome Surveillance in Live Cells/ Genome Surveillance**

Name Project Leader(s):

Prof. Roland Kanaar
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Project-related website: -

Reporting period: from 01/03/2004 to 01/03/2007 (Please report for the whole duration of your project)

List all Principal Investigators (PIs), Associated Partners (APs) and Co-operating Partners (CPs) of the CRP

PI 1
name: **Dr. Wim Vermeulen**
PI 2
name: **Prof. Alan R. Lehmann**
PI 3
name: **Prof. Dr. Jiri Bartek**
PI 4

name: **Dr. Jiri Lukas**
PI 5
name: **Prof. Dr. Leon H. F. Mullenders**
PI 6
name: **Dr. Adriaan. B. Houtsmuller**
PI 7
name: **Prof. Dr. Thomas Cremer**
PI 8
name: **Dr. Anna A. Friedl**
PI 9
name: **Prof. Dr. Günther Dollinger**

A.3. Report of the CRP in terms of Networking, Training and Dissemination

→ (To be completed by Project Leader with input from CRP members)

Please provide a concise and clear description of the networking, training and dissemination activities undertaken by this CRP, addressing the following issues:

6. Networking activities: Of all the networking activities developed during this period, please highlight the most important one for this CRP (in terms of outcome, impact, opportunities for trans-national collaborations and synergy with other European and international initiatives)

Scientific collaboration and development of FRAP analysis platform between Vermeulen and Houtsmuller

Scientific collaboration and analysis of TLS-factors between Alan Lehmann and Vermeulen

Scientific collaboration and analysis of TLS-factors between Alan Lehmann and Bartek

Scientific collaboration between Vermeulen and Mullenders on UV-DDB activity

Scientific collaboration between Vermeulen and Lukas on localised DNA damage induction in relation to XPC damage sensing

Scientific collaboration and training between Lehmann and Adriaan Houtsmuller

Scientific collaboration between Bartek and Lehmann

We have been directly collaborating with the following laboratories/institutions:

Stephen Jackson; University of Cambridge, UK (spatio-temporal regulation of ATM and ATR).

Michael Kastan; St. Jude Childern's hospital, Memphis. TN, USA (nuclear localization of checkpoint regulators).

Michele Pagano; New York Universtiy School of Medicine, NY, USA (claspin proteolysis).

Initiation of collaboration with drs. J van Noort, C. Logie and A. Brehm from other CRP: generation of nucleosomal arrays for in vitro investigation of transcription initiation by steroid receptors

Collaboration with dr. R. Renkavitsj: interaction of CTCF with steroid receptors

Hosting PhD-students of dr. P. Hozak and R. Renkavitsj from two other CRPs for an in vivo imaging course.

Activities have led to interaction with other research programmes within EU: e.g. EU integrated Project RiscRad (F16R-CT-2003-508842), DNA Repair (LSHC-CT-2005-512113), Marie Curie RTN (MRTN-CT-

2003-503618) and 03-DYNA-F-19 (van Haaften et al, 2006).

Via these interactions and collaborative meetings we have established collaborations with research groups outside the EU, mainly in the US (A. Tomkinson, O. Scharer).

7. Training activities: Please, highlight the most useful training activity (workshop, course or school) undertaken by senior or junior researchers of this CRP

Scientific training between Lehmann and Wim Vermeulen

Personnel from the groups of Dollinger/Friedl visited the group of R. Kanaar (same dept. as WP1, Vermeulen) for training in producing cells expressing fluorescent proteins and handling them for live cell microscopy.

One major training activity was the workshop on 'DNA repair: from Molecular Mechanism to human Disease', Noordwijkerhout, The Netherlands, 2006. Most groups within this CRP participated on this workshop, which had a well organized and stimulating poster session and used novel tools for student education such as assigned tasks for students to report on discussions during the meeting.

8. Dissemination activities: What has this CRP done to promote the EuroDYNA Programme more actively and widely? Please, indicate the outcome and impact of the dissemination activities developed during this period

We are currently collaborating with other member of the CRP within the EuroDYNA program in order to establish a common platform for studying protein mobility both in vivo and in silico. Results from studies of different repair factors are used to create and improve a database that could then be used to calculated mobility parameters of future proteins of interest.

Furthermore, the results and concepts derived from this work have been disseminated on conferences and courses for Ph.D. students and young scientists as detailed in the appendix. In addition, exchange of personnel between the WPs has occurred. Scientists involved in WP7 will take part in two DFG-funded Excellence Cluster programs, starting from November 2006.

Interactions have occurred with other research programmes: EU Integrated Projects RISC-RAD (DNA Damage Repair, Genomic Instability and Radiation Induced Cancer, FI6R-CT-2003-508842), DNA Repair(DNA Damage Response and Repair Mechanisms), and Marie Curie RTN MRTN-CT-2003-503618

Results have been presented to general public (see publication Cicero, Leiden). Several guided tours for guest scientists from Germany and Europe allowed to demonstrate the potentials of experiments using the ion microbeam to a broader public. Also the microbeam facilitiy has been presented on a public university day in Okt. 2006, and attracted approximately 820 visitors.

A.4 Valuable feedback from this CRP

→ (To be completed by Project Leader with input from CRP members)

9. Key contribution of this CRP to the EURODYNA Programme: How has the EuroDYNA Programme benefited from this CRP? Please, provide one example that clearly illustrates your valuable input (whether scientific, networking, training and/or dissemination input)

There are two key contributions:

1. Development of protocols to rigorously analyze FRAP data from live cell imaging.
2. Development of methods to locally induce diiferent kinds of DNA damage living cells.
3. Information on dynamic properties of DNA damage respoknse proteins.
4. Via the interconnection with another CRP, novel factors were identified.

10. Key contribution of the EURODYNA Programme to this CRP: How has your participation in the EuroDYNA Programme influenced your research (in terms of visibility, collaborations, opportunities, ideas)

The EuroDYNA programme has provided a significant contribution to the execution of a part of the research program of the CRP. It has intisified collaborations between PI's 1, 2, 4, 5 and 6 (see 2 and publications). The stimulating scientific discussions at previous EuroDYNA meetings have contributed to important intellectual input.

For the group of PI 9 it was a very usefull participation because it realized the idea of strong interaction between biophysics and physics group. It was the starting point for the installation of a successful irradiation facility for radiobiology/physics experiments. It has become the most visible European installation for live cell irradiation by energetic ions. It was evaluated to be the most experienced facility for ion beam irradiation across Europe (evaluated within a newly started I3 initiative within FP7 program between 24 ion beam labs in Europe)

A.5 Follow up related to the CRP and EuroDYNA Programme

→ (To be completed by Project Leader with input from CRP members)

11. Follow up: Please provided details of the most important new initiatives (either within a national or an international context) that have been developed as a result of the collaboration of this CRP and the EuroDYNA Programme.

- Capturing DNA repair complexes for structural studies. This approach uses 3-D structure analysis and cryo-EM (in collaboration with Leiden University, The Netherlands).
- Analysis of the DNA damage signaling pathways related to nucleotide excision repair.
- Detailed analysis of TLS-polymerase in NER (PI2 with PI5).

- Analysis of the protein dynamics and reaction kinetics of factors at the cross road between different DNA repair pathways and DNA replication.

- Analysis of UV-induced chromatin modifications (PI1 with PI3/4).

- The CRP was the starting point for the access of the G. Dollinger and A. Friedl in to the center of excellence MAP (Munich center for advanced photonics) which has started in Nov. 2006 and which has decided to be supported from the German science foundation DFG for the most successful research activities at the German universities.

- It is also the starting point for taking part in an I3 initiative (submitted within call of the European FP 7 program) of 11 ion beam labs across Europe where ion beam analysis and energetic ion irradiation facilities will be opened to potential European users within a common enterprise.

A. 5. List of Publications, Dissemination and Outreach Activities undertaken by the CRP

→ (To be completed by Project Leader with input from CRP members)

Publications

- Articles

Peer reviewed articles in journals (published, in press or submitted)

1. S. Bergink, F.A. Salomons, D. Hoogstraten, T.A. Groothuis, H. de Waard, J. Wu, L. Yuan, E. Citterio, A.B. Houtsmuller, J. Neefjes, J.H. Hoeijmakers, W. Vermeulen and N.P. Dantuma. DNA damage triggers nucleotide excision repair-dependent monoubiquitylation of histone H2A, Genes Dev 20 (2006) 1343-1352.

2. J. Essers, W. Vermeulen and A.B. Houtsmuller DNA damage repair: anytime, anywhere?, Curr Opin Cell Biol 18 (2006) 240-246.

3. G. Giglia-Mari, C. Miquel, A.F. Theil, P.O. Mari, D. Hoogstraten, J.M. Ng, C. Dinant, J.H. Hoeijmakers and W. Vermeulen. Dynamic Interaction of TTDA with TFIIH Is Stabilized by Nucleotide Excision Repair in Living Cells, PLoS Biol 4 (2006) e156.

4. E.A. Maltseva, N.I. Rechkunova, I.O. Petruseva, V.N. Silnikov, W. Vermeulen and O.I. Lavrik. Interaction of nucleotide excision repair factors RPA and XPA with DNA containing bulky photoreactive groups imitating damages, Biochemistry (Mosc) 71 (2006) 270-278.

5. S. Bergink, L.A. Severijnen, N. Wijgers, K. Sugasawa, H. Yousaf, J.M. Kros, J. van Swieten, B.A. Oostra, J.H. Hoeijmakers, W. Vermeulen and R. Willemsen The DNA repair-ubiquitin-associated HR23 proteins are constituents of neuronal inclusions in specific neurodegenerative disorders without hampering DNA repair, Neurobiol Dis 23 (2006) 708-716.

6. J.O. Andressoo, J.R. Mitchell, J. de Wit, D. Hoogstraten, M. Volker, W. Toussaint, E. Speksnijder,

R.B. Beems, H. van Steeg, J. Jans, C.I. de Zeeuw, N.G. Jaspers, A. Raams, A.R. Lehmann, W. Vermeulen, J.H. Hoeijmakers and G.T. van der Horst An Xpd mouse model for the combined xeroderma pigmentosum/Cockayne syndrome exhibiting both cancer predisposition and segmental progeria, Cancer Cell 10 (2006) 121-132.

7. L.J. Niedernhofer, G.A. Garinis, A. Raams, A.S. Lalai, A.R. Robinson, E. Appeldoorn, H. Odijk, R. Oostendorp, A. Ahmad, W. van Leeuwen, A.F. Theil, W. Vermeulen, G.T. van der Horst, P. Meinecke, W.J. Kleijer, J. Vijg, N.G. Jaspers and J.H. Hoeijmakers A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis, Nature 444 (2006) 1038-1043.

8. A. Zotter, M.S. Luijsterburg, D.O. Warmerdam, S. Ibrahim, A. Nigg, W.A. van Cappellen, J.H. Hoeijmakers, R. van Driel, W. Vermeulen and A.B. Houtsmuller Recruitment of the nucleotide excision repair endonuclease XPG to sites of UV-induced dna damage depends on functional TFIIH, Mol Cell Biol 26 (2006) 8868-8879.

9. N.G. Jaspers, A. Raams, M.C. Silengo, N. Wijgers, L.J. Niedernhofer, A.R. Robinson, G. Giglia-Mari, D. Hoogstraten, W.J. Kleijer, J.H. Hoeijmakers and W. Vermeulen First case of human ERCC1 deficiency has cerebro-oculofacio-skeletal syndrome with a mild defect in nucleotide excision repair and severe developmental failure., Am J Hum Genet 80 (2007); 457-466.

10. S. Bergink, N.G. Jaspers and W. Vermeulen Regulation of UV-induced DNA damage response by ubiquitylation,DNA Repair 6 (2007); 1231-1242.

11. M.S. Luijsterburg MS, J. Goedhart, J. Moser, H. Kool, B. Geverts, A.B. Houtsmuller, L.H. Mullenders, W. Vermeulen, R. van Driel. Dynamic in vivo interaction of DDB2 E3 ubiquitin ligase with UV-damaged DNA is independent of damage-recognition protein XPC. J. Cell Sci. 120 (2007); 2706-2716.

12. C. Dinant, M. de Jager, J. Essers, W.A. van Cappellen, R. Kanaar, A.B. Houtsmuller, W. Vermeulen. Activation of multiple DNA repair pathways by sub-nuclear damage induction methods. J. Cell Sci. 120 (2007); 2731-2740.

13. F. Nicassio, N. Corrado, J.H. Vissers, L.B. Areces, S. Bergink, J.A. Marteijn, B. Geverts, A.B. Houtsmuller, W. Vermeulen, P.P. Di Fiore, E. Citterio. Human USP3 is a chromatin modifier required for S phase progression and genome stability. Curr. Biol. 17 (2007); 1972-1977.

14. E.A. Maltseva, N.I. Rechkunova, I.O. Petruseva, W. Vermeulen, O.D. Schärer and O.I. Lavrik. Crosslinking of nucleotide excision repair proteins with DNA containing photoreactive damages. Bioorganic Chemistry (2008) *in press.*

15. C. Dinant, M. van Royen, W. Vermeulen and A.B. Houtsmuller. Measuring Fluorescence Resonance Energy Transfer in Living Cells from GFP to YFP with Spectral Imaging and Quantitative Acceptor Photobleaching. J. of Microscopy (2008) *in press*.

16. D. Hoogstraten, S.Bergink, V. Verbiest, M. Luijsterburg, B. Geverts, A. Raams, C. Dinant, J. H. J. Hoeijmakers, W. Vermeulen (corresponding author) and A.B. Houtsmuller. DNA-damage

17. sensing by xeroderma pigmentosum group C in living cells. J. of Cell Sci. (2008) in press.

18. Lehmann AR, Niimi A, Ogi T, Brown S, Sabbioneda S, Wing JF, Kannouche PL, Green CM.

    Translesion synthesis: Y-family polymerases and the polymerase switch. DNA Repair (Amst). 2007, 6:891-9.

19. Mailand, N., Bekker-Jensen, S., Faustrup, H., Melander, F., Bartek, J., Lukas, C., and Lukas, J. The RNF8 ubiquitin ligase promotes assembly of repair proteins at the DNA damage-modified chromatin. *Cell,* 131, 887-900 (2007).

20. Bekker-Jensen, S., Fugger, K., Danielsen, J. R., Gromova, I., Celis, J., Bartek, J., Lukas, J., and Mailand, N. Human Xip1 (C2orf13) is a novel regulator of cellular responses to DNA strand breaks. *J. Biol. Chem.,* 282, 19638-19643 (2007).

21. Melander, F., Bekker-Jensen, S., Falck, J., Bartek, J., Mailand, N., and Lukas, J. Phosphorylation of MDC1 by casein kinase 2 mediates retention of NBS1 at the DNA damage-modified chromatin. *J. Cell Biol.,* in press (2007).

22. Löffler, H., Rebacz, B., Ho, A. D., Lukas, J., Bartek, J., and Krämer A. Chk1-dependent regulation of Cdc25B activity functions to coordinate mitotic events. *Cell Cycle,* 5, 2543-2547 (2007).

23. Löffler, H., Bochtler, T., Fritz, B., Tews, B., Ho, A. D., Lukas, J., Bartek, J., and Krämer, A. DNA dmage-induced accumulation of centrosomal Chk1 contributes to its checkpoint function. *Cell Cycle* 6, 2541-2548 (2007).

24. Bartek J., and Lukas, J. DNA damage checkpoints: From initiation to recovery or adaptation. *Curr. Opin. Cell Biol.,* 19, 238-245 (2007).

25. Fousteri M, Mullenders LH. Transcription-coupled nucleotide excision repair in mammalian cells: molecular mechanisms and biological effects. Cell Res, 2008 18 (1), 73-84. Review.

26. Jill Moser, Hanneke Kool, Saskia Lagerwerf, Keith Caldecott, Leon H.F. Mullenders, Maria I. Fousteri. Gap filling in mammalian nucleotide excision repair involves DNA polymerase δ /XRCC1-DNA Ligase IIIa and an S-phase dependent utilisation of DNA polymerase ε/DNA ligase I. Mol Cell, 2007 6, 1642-1650.

27. Martijn S. Luijsterburg , Joachim Goedhart , Jill Moser, Hanneke Kool , Bart Geverts , Adriaan B. Houtsmuller, Leon H.F. Mullenders,Wim Vermeulen and Roel van Driel. DDB2 E3 ubiquitin ligase interacts with UV-damaged DNA independently of damage recognition protein XPC in living cells. J Cell Sci, 2007 120, 2706-2716.

28. Vrouwe MG, Elghalbzouri-Maghrani E, Meijers M, Schouten P, Godthelp BC, Bhuiyan ZA, Redeker EJ, Mannens MM, Mullenders LH, Pastink A, Darroudi F. Increased DNA damage sensitivity of Cornelia de Lange syndrome cells: evidence for impaired recombinational repair. Hum Mol Genet , 2007 16, 1478-87.

29. Fousteri M, Vermeulen W, van Zeeland AA, Mullenders LH Cockayne syndrome A and B proteins differentially regulate recruitment of chromatin remodeling and repair factors to stalled

RNA polymerase II in vivo. Mol Cell, 2006 23, 71-82.

30. van Haaften G, Romeijn R, Pothof J, Koole W, Mullenders LH, Pastink A, Plasterk RH, Tijsterman M. Identification of conserved pathways of DNA-damage response and radiation protection by genome-wide RNAi. Curr Biol, 2006 16, 1344-50.

31. van der Wees C, Jansen J, Vrieling H, van der Laarse A, Van Zeeland A, Mullenders L. Nucleotide excision repair in differentiated cells. Mutat Res, 2007 Jan 3, 614 (1-2), 16-23.

32. de Gruijl FR, Mullenders LH, Stout GJ. Elimination of keratinocytes stagnant in s-phase through epidermal turnover instead of in situ situ apoptosis. Cell Cycle, 2006 5, 565-6, 2006.

33. Alekseev S, Kool H, Rebel H, Fousteri M, Moser J, Backendorf C, de Gruijl FR, Vrieling H, Mullenders LH. Enhanced DDB2 expression protects mice from carcinogenic effects of chronic UV-B irradiation. Cancer Res, 2005 65,10298-306.

34. Theron T, Fousteri MI, Volker M, Harries LW, Botta E, Stefanini M, Fujimoto M, Andressoo JO, Mitchell J, Jaspers NG, McDaniel LD, Mullenders LH, Lehmann AR. Transcription-associated breaks in xeroderma pigmentosum group D cells from patients with combined features of xeroderma pigmentosum and Cockayne syndrome. Mol Cell Biol, 2005 25, 8368-78.

35. Fousteri M, van Hoffen A, Vargova H, Mullenders LH. Repair of DNA lesions in chromosomal DNA: impact of chromatin structure and and Cockayne syndrome proteins. DNA Repair, 2005 4, 919-25. Review.

36. Moser J, Volker M, Kool H, Alekseev S, Vrieling H, Yasui A, van Zeeland AA, Mullenders LH. The UV-damaged DNA binding protein mediates efficient targeting of the nucleotide excision repair complex to UV-induced photo lesions. DNA Repair, 2005 2,4, 571-82.

37. Alex Pines, Claude Backendorf, Sergey Alekseev, Jacob G. Jansen, Frank R. de Gruijl, Harry Vrieling, and Leon H.F. Mullenders. Differential repair of UV photoproducts in murine keratinocytes and fibroblasts is mediated by differential activity of UV-DDB, submitted for publication.

38. Greubel C, Hable V, Drexler GA, Hauptner A, Dietzel S, Strickfaden H, Baur I, Krücken R, Cremer T, Friedl AA, Dollinger G (2008) Quantitative Analysis of DNA Damage Response Factors after Sequential Ion Microirradiation. Radiat Env Biophys (submitted).

39. Greubel C, Hable V, Drexler GA, Hauptner A, Dietzel S, Strickfaden H, Baur I, Krücken R, Cremer T, Dollinger G, Friedl AA (2008) Competition effect in DNA damage response. Radiat Env Biophys (submitted).

40. Hauptner, A., Krücken, R., Greubel, C., Hable, V., Dollinger, G., Drexler, G.A., Deutsch, M., Löwe, R., Friedl, A.A., Dietzel, S., Strickfaden, H., Cremer, T (2006) DNA repair protein distribution along the tracks of energetic ions. Radiat Prot Dosim 122:147-149.

41. Hauptner, A., Friedland, W., Dietzel, S., Drexler, G.A., Greubel, C., Hable, V., Strickfaden, H., Cremer, T., Friedl, A.A., Krücken, R., Paretzke, H.G., Dollinger. G (2006) Spatial distribution of

DNA double strand breaks from ion tracks. Mat Fys Medd Dan Vid Selsk 52: 59-85.

42. Dollinger, G., Bergmaier, A., Hauptner, A., Dietzel, S., Drexler, G.A., Greubel, C., Hable, V., Reichart, P., Krücken, R., Cremer, T., Friedl, A.A (2006) Hydrogen microscopy and analysis of DNA repair using focused high energy ion beams. Nuclear Instruments and Methods in Physics Research B 249:270-277.

43. Hable, V., Dollinger, G., Greubel, C., Hauptner, A., Krücken, R., Dietzel, S., Cremer, T., Drexler, G.A., Friedl, A.A., Löwe, R (2006) Methods for quantitative evaluation of dynamics of repair proteins within irradiated cells. Nuclear Instruments and Methods in Physics Research B 245:298-301.

44. Dollinger, G., Hable, V., Hauptner, A., Krücken, R., Reichart, P., Friedl, A.A., Drexler, G., Cremer, T., Dietzel, S (2005) Microirradiation of cells with energetic heavy ions. Nuclear Instruments and Methods in Physics Research B 231:195-201.

Published contributions to international conferences

1. C. Greubel, V. Hable, G. Dollinger, A. Hauptner, R. Krücken, H., Strickfaden, S. Dietzel, T. Cremer, G. A. Drexler, M. Deutsch, A. A. Friedl. The Munich Microprobe SNAKE, a Single-Ion Cell Irradiation Facility. Radiation Research 166, 654 (2006).

2. A.A. Friedl, G. A. Drexler, M. Deutsch, H. Strickfaden, S. Dietzel, T., Cremer, A. Hauptner, R. Krücken, C. Greubel, V. Hable and G. Dollinger. Radiobiological Experiments at the Munich Microprobe SNAKE. Radiation Research 166 668-669 (2006).

3. V. Hable, G. Dollinger, C. Greubel, A. Hauptner, R. Krücken, S. Dietzel, T. Cremer, G. A. Drexler , A. A. Friedl. Dynamics of DNA Repair Proteins after Directed Heavy-Ion Cell Irradiation. Radiation Research 166 676 (2006).

4. Friedl, A.A., Drexler, G.A., Löwe, R., Dollinger, G., Hauptner, A., Hable, V., Greubel, C., Krücken, R., Cremer, T., Dietzel, S. Radiobiological experiments at the Munich ion microbeam SNAKE. Proceedings of the 9th International Conference on Health Effects of Incorporated Radionuclides. GSF-Bericht 06/05 (2005).

5. DYNAMICS OF DNA REPAIR PROTEINS AFTER DIRECTED HEAVY-ION CELL IRRADIATION. V. Hable, G. Dollinger, C. Greubel, A. Hauptner, R. Krücken, S. Dietzel, T. Cremer, G. A. Drexler and A. A. Friedl, Radiation Research 166 (2006) 676.

Presentations in Scientific Meetings

- Oral presentations (indicate invited / keynote talks)

Presentations PI 1 (all invited)

EMBO/FEBS conference on Nuclear Structure and Dynamics, (2005, La Grande Motte, France); Gordon Research Conference, DNA Damage, Mutation and Cancer, (2006, Ventura, CA, USA); DNA Repair: from Molecular Mechanism to Human Disease, (2006, Noordwijkerhout , The Netherlands); Erling Seeberg Symposium on DNA repair, (2006, Lofoten, Norway); DNA repair: Molecules to Patients (2006, Landsdowne, VA, USA); EU-FP6 meeting on DNA repair disorders (2006, Marseille, France); Gordon Research Conference, Mammalian DNA Repair (2007, Ventura, CA, USA). Joint EU-USA DNA repair workshop (2007, Berkeley, CA, USA). Translating the Cancer Genome (2007, Helsinki, Finland). EMBO conference series on Nuclear Structure and Dynamics (2007, Montpellier, France). International Congress on Biophotonics (2008, Sacramento, CA, USA).

Presentations PI 2 (all invited):

A. Lehmann: Gordon Research Conference on mutagenesis, USA, 2006; DNA Repair: from Molecular Mechanism to Human Disease, (2006, Noordwijkerhout , The Netherlands); Gordon Research Conference on Radiation Oncology, USA, 2008; Gordon Research Conference, Mammalian DNA Repair (2007, Ventura, CA, USA). Joint EU-USA DNA repair workshop (2007, Berkeley, CA, USA). Dutch Radiobiology and genetic toxicology meeting, 2007.

Project work presented by S. Sabbioneda at:

Third EU-RTN meeting, 2006, Marseille, France. Dynamics of TLS polymerases; Fourth RTN meeting, 2007, Strasbourg, France. Dynamics of TLS polymerases; EuroDYNA Conference 2006, Brno, Czech Republic. Dynamics of translesion synthesis polymerases in living cells.; Joint EU-USA DNA Repair Workshop, Berkeley, USA, 2007. Dynamics of translesion synthesis polymerases in living cells; Abcam Maintenance of Genome Stability, Puerto Vallarta, Mexico, 2008. Dynamics of translesion synthesis polymerases in living cells.

Presentations PI 3/4:

Participation in international conferences, symposia, seminars etc.

Title of event	Venue	Name(s) of participant(s)	Contribution (talk, abstract, paper, poster, other)	Invited talk (please check)
Translating the Cancer Genome	Helsinki, FI	Jiri Lukas		Keynote speaker
Links Between Cancer, Replication Stress and Genomic Integrity	Madrid, ES	Jiri Lukas		Keynote speaker

Institute of Molecular Cancer Research	Zürich, CH	Jiri Lukas		x
Gurdon Institute	Cambridge, UK	Jiri Lukas		x
Huthison MRC Centre	Cambridge, UK	Jiri Lukas		x
University of Cambridge	Cambridge, UK	Jiri Lukas		x
Keystone Conference	Denver, USA	Jiri Bartek		x
Czech Academy of Sciences, 'DNA damage response: Mechanisms and relevance for human cancer'	Prag, CZ	Jiri Bartek		Keynote speaker
Oxford University Radiology Institute, 'DNA damage response: Mechanisms and relevance for human cancer'	Oxford, UK	Jiri Bartek		Keynote speaker
German Cancer Congress	Frankfurt , DE	Jiri Bartek		x
DNA Repair Meeting	San Francisco, USA	Jiri Bartek		x
American Association For Cancer Congress	Los Angeles, USA	Jiri Bartek		x
Molecular Pathology Workshop	Olomouc, CZ	Jiri Bartek		x
Radiation Symposium	Wolfsberg, CH	Jiri Bartek		x
DGF China/Denmark Symposium	Bejing, CN	Jiri Bartek		x
Seminar, University of Barcelone, 'DNA damage response: Mechanisms and relevance for human cancer'	Barcelone, ES	Jiri Bartek		Keynote speaker
Gordon Conferesce	Boston, USA	Jiri Bartek		x
FEBS Congress	Vienna, AT	Jiri Bartek		x
Molecular Biology Symposium, Göttingen University	Göttingen, SW	Jiri Bartek		x

Munich University	Munich, DE	Jiri Bartek		x
Karolinska Institute, 'DNA damage response: Mechanisms and relevance for human cancer'	Stockholm, SW	Jiri Bartek		Keynote speaker
Swiss DNA Repair meeting	Bern, CH	Jiri Bartek		x
EMBO Workshop	Athens GR	Jiri Bartek		x
Nature Cancer Conference	Capri, IT	Jiri Bartek		x
CNIO Meeting	Madrid, ES	Jiri Bartek		x
p53 Workshop	Lyon, FR	Jiri Bartek		x
University of St. Andrews Symposium	Edinburg, UK	Jiri Bartek		x
Institute of Molecular Genetics, 'DNA damage response: Mechanisms and relevance for human cancer'	Prag CZ	Jiri Bartek		Keynote speaker
Breast Cancer Symposium	Rome, IT	Jiri Bartek		x
Keystone meeting on Autophagy and cell death	International conference, USA	Marja Jäättelä, Maria Høyer-Hansen	x	x
EMBO meeting on Membrane Trafficking	International conference, AT	Marja Jäättelä	x	x
EMBO meeting on stress and molecular chaperones	International conference, PT	Mikkel Rohde		x

Presentattions PI 5 (all invited):

M. Fousteri: Stalling of transcription as a sensor for DNA damage responses. (Basel, September 2007); DNA damage–stalled RNA polymerase and the assembly of repair factors for transcription coupled repair. Gordon Research Conference, DNA damage, mutation and cancer, (Ventura, March 2006); DNA damage recognition and complex assembly in expressed chromatin. Third Dutch Chromatin Community Meeting, (Rolduc, Netherlands, 7-8 December, 2005)

L. Mullenders: Stem cells: from DNA damage repair to their role in skin carcinogenesis. Cell-fate decision: Function and dysfunction in host defense – Tumor, Infection and Immunity. International symposium: Joint

Symposium of the 3rd International Symposium of Institutes Network and Hot Spring Harbor-Global COE Symposium, (Beppu, Japan, February 2008); Assembly of repair and chromatin remodeling factors for TCR. Mammalian DNA repair, Gordon Research Conference, (Ventura, 2007); DNA repair: from past to future. European Strategy Photobiology, (Bath, UK, September 2007 [Keynote]); L. Mullenders. DNA damage, DNA repair and biological effects. CECAM, (Lyon, France, December 2007); Assembly of NER complexes in vivo. DNA Repair: from Molecular Mechanism to Human Disease. (Noordwijkerhout, April 2-7, 2006); From initial DNA damage to cancer: Lessons from mouse models. Cancer 2006: From molecular biology processes to tumor-tailored therapy. Congress Centre Academia, (Stara Lesna, Slovakia. 20th - 24th August, 2006); Differential roles of Cockayne syndrome A and B proteins in the assembly of the transcription coupled repair complex. Workshop on Xeroderma pigmentosum and other diseases of human premature aging and DNA repair: molecules to patients. (Washington DC, September 5-8, 2006); DNA lesion recognition and assembly of repair complexes in expressed and non-expressed chromatin. 2nd EU-US meeting on DNA Repair. (Erice, Italy November 29 - December 3, 2005); From Initial DNA Damage to Cancer: Lessons from Mouse Models ICEM (Seattle, Washington, August 31- September 2, 2005). Assembly of repair factors for transcription coupled repair. 9th International Conference on Environmental Mutagens, San Francisco, September 3-8, 2005.

Presentations PI 9.

G. Dollinger: “Live cell microirradiation using energetic ions”, Plenary invited talk at IMMM (International conference on Ion Beam Modification of Materials), (September 2008, Dresden Germany); V. Hable, “Microirradiation and online live cell experiments at SNAKE”, invited talk at ICNMTA (Cnternational Conference on Nuclear Microprobe Technology and Application),(July, 2008, Debrecen, Ungarn); C. Greubel, “Cell Irradiation at the Ion Microprobe SNAKE”, invited talk, IBA18 (International conference on Ion Beam Analysis), (September 2007, Hyderabad, India); V. Hable: “Microirradiation of cells by energetic ions, invited talk at “IBIBAM - Ion Beams in Biology and Medicine (Radioonkologie)”, (September 2007, Heidelberg); G. Dollinger:” Irradiation of single cells with protons and ions”, invited talk at International symposium: Protons, Ions and Neutrons in Radiation Oncology”, (July 2007, Munich, Germany); G. Dollinger, “Questions on ion-matter interaction in radiation biology (cellular level)”, invited talk at Ion06, (Juni 2006, Kopenhagen, Denmark); G. Dollinger, “Radiobiological Experiments at the Munich Microprobe SNAKE”, invited talk at ICNMTA (Cnternational Conference on Nuclear Microprobe Technology and Application), (July 2006, Singapore).

Public Outreach:

- National / international Newspaper articles (presenting your CRP or part of your work)

PI 3/4: *Danish press*: Article in ***Weekendavisen*** (reporting on the discovery of the RNF8 ubbiquitin ligase and the strategy to monitor nuclear dynamics of the DNA damage response in living cells).

- TV appearance

PI 3/4: ***Danish TV***: Science section in the daily news series ***‘Deadline’*** reporting on the dynamics of DNA

repair factors and their role in preventing diseases associated with genomic instability.

- Radio appearance

- Other (*please define*)

For the general public (in Dutch language):

L. Mullenders: Fingerprint van toxische stoffen. C2W Life Sciences 16, 7 (2007).

L. Mullenders. Genetische reparaties op het spoor. Cicero 10, Sept 2006. LUMC, Leiden.

Other Activities / Products

- Patents

- Websites

www.genotoxic.dk; continuous updating of research news, public outreach and educational activity of the Lukas/Bartek laboratories.

IMPORTANT: In your list, please identify with an asterisk (*) those publications and contributions in which the EuroDYNA Programme is properly acknowledged.

Nuclear action of miRNAs

Abstract

Double stranded RNA (dsRNA) is potent inducer of gene silencing. The mechanism by which these molecules induce silencing is evolutionary conserved, and represents a very powerful and specific way of gene activity control. One of the intermediates of this silencing process is a short RNA(Srna) molecule that has been named short interfering RNA (siRNA) or micro RNA (miRNA). These molecules act as guides for either an RNA degradation enzyme that is active in the cytosol or a complex that targets translation inhibition. In addition to these cytosolic events, nuclear effects of dsRNA have also been observed. In plants, dsRNA leads to methylation of homologous DNA sequences, and induces transcriptional silencing when promoter DNA is targeted. In yeast, dsRNA mediated processes have been implicated in centromere function. In animals, evidence for such nuclear effects has been obtained as well. For example, phenotypes of C. elegans mutants defective in RNAi suggest an impaired centromere function, and partially overlap with phenotypes associated with defects in the maintenance of silent chromatin states. In this research proposal we aim at a better understanding of the nuclear effects of dsRNA. We will do this by analyzing nuclear dsRNA processing, by identifying nuclear sRNA, by analyzing proteins associating with the nuclear sRNA and by analyzing the effects of nuclear sRNA on chromatin modifications and transcriptional activity.

Partners

(NWO, FWF)

Dr René F. Ketting (Project Leader)
Netherlands Institute for Developmental Biology, Utrecht, The Netherlands

Dr Majori Matzke
Austrian Academy of Sciences, Vienna, Austria

Part A. Collaborative Research Project (CRP)

→ (To be completed by Project Leader)

A.1. General information about the Collaborative Research Project (CRP)
CRP Number: 03-DYNA–F-19 **CRP Title and Acronym**: **Nuclear action of microRNAs / NuMi** **Name Project Leader**: Dr. René Ketting **Project-related website**: www.niob.knaw.nl/researchpages/ketting/index.html **Reporting period**: from 01/10/2005 to 01/10/2008 (Please report for the whole duration of your project)
List all Principal Investigators (PIs), Associated Partners (APs) and Co-operating Partners (CPs) of the CRP
PI 1
name: **Dr. René F. Ketting**
PI 2
name: **Dr. Marjori Matzke**

A.3. Report of the CRP in terms of Networking, Training and Dissemination

→ (To be completed by Project Leader with input from CRP members)

Please provide a concise and clear description of the networking, training and dissemination activities undertaken by this CRP, addressing the following issues:

6. Networking activities: Of all the networking activities developed during this period, please highlight the most important one for this CRP (in terms of outcome, impact, opportunities for trans-national collaborations and synergy with other European and international initiatives)

The most directly useful networking activity was a meeting of both CRP groups in Vienna, when it started to become clear in what animal systems signs of RNA mediated chromatin changes were evident. This took place in Vienna, in December 2007.

7. Training activities: Please, highlight the most useful training activity (workshop, course or school) undertaken by senior or junior researchers of this CRP

NA

8. Dissemination activities: What has this CRP done to promote the EuroDYNA Programme more actively and widely? Please, indicate the outcome and impact of the dissemination activities developed during this period

Both groups have acknowledged the EuroDYNA programme in their research papers, and always acknowledge it in presentations.

A.4 Valuable feedback from this CRP

→ (To be completed by Project Leader with input from CRP members)

Please, provide a concise and clear answer to these questions:

9. Key contribution of this CRP to the EURODYNA Programme: How has the EuroDYNA Programme benefited from this CRP? Please, provide one example that clearly illustrates your valuable input (whether scientific, networking, training and/or dissemination input)

This CRP has provided EuroDYNA with clear examples of how and which RNA molecules in the nucleus can effect gene activity in a direct manner; e.g. by directly interacting with the transcriptional activity of genes or regions of the genome. This sort of role for RNA has so far been largely neglected, especially in

animal systems. This, together with the upcoming notion that large regions of the genome that were previously thought to be transcriptionally silent are actually producing lots of non-coding RNAs, makes that RNA mediated effects on chromatin have to be taken seriously in many systems.

10. Key contribution of the EURODYNA Programme to this CRP: How has your participation in the EuroDYNA Programme influenced your research (in terms of visibility, collaborations, opportunities, ideas)

EuroDYNA has clearly intensified the contacts between the two partners in our CRP. Also in future research this will surely proof to be a valuable thing for both laboratories. This interaction has surely influenced the ways we were thinking about potential roles for RNA molecules in chromosome dynamics, and will make future collaborations much more easy to start. As already indicated, the link between small RNA and chromosome in the animal systems studied are just starting to become clear, so most likely interactions between both labs in the future will be more intense than those during the EuroDYNA funding period itself.

A.5 Follow up related to the CRP and EuroDYNA Programme

→ (To be completed by Project Leader with input from CRP members)

11. Follow up: Please provided details of the most important new initiatives (either within a national or an international context) that have been developed as a result of the collaboration of this CRP and the EuroDYNA Programme.

One specific idea that has come from this CRP that is currently being followed up in both labs constituting the CRP is on the role of protein clamps stabilizing either RNA-DNA or RNA-RNA interactions. This is based on the genetic identification of an SMC-like protein in Arabidopsis, required for RNA directed DNA methylation. In parallel, an SMC-like protein was found to interact with a Piwi protein in the zebrafish; specifically, with the Piwi protein that is also found within he nucleus, zili.

A. 6. List of Publications, Dissemination and Outreach Activities undertaken by the CRP

→ (To be completed by Project Leader with input from CRP members)

Publications

- Articles

Peer reviewed articles in journals (published, in press or submitted)

*Huettel B, Kanno T, Daxinger L, Aufsatz W, Matzke AJM, Matzke M (2006) Endogenous targets of RNA-directed DNA methylation and Pol IV in Arabidopsis. **EMBO J** 25: 2828-2836.

*Houwing S., Kamminga L.M., Berezikov E., Cronembold D., Girard A., van den Elst H., Filippov D.V., Blaser H., Raz E., Moens C.B., Plasterk R.H., Hannon G.J., Draper B.W., Ketting R.F. (2007)

A role for Piwi and piRNAs in germ cell maintenance and transposon silencing in Zebrafish. **Cell** 29:69-82

*Kanno T, Bucher E, Daxinger L, Huettel B, Böhmdorfer G, Gregor W, Kreil D, Matzke M, Matzke AJM (2008) An SMC hinge domain containing protein is required for RNA-directed DNA methylation. **Nature Genetics**, in press.

Published contributions to international conferences

News & Views-type articles

Ketting, R.F. (2006) Partners in Dicing.**Genome Biol**. 7: 210

*Huettel B, Kanno T, Daxinger L, Bucher E, van der Winden J, Matzke AJM, Matzke M (2007) RNA-directed DNA methylation mediated by DRD1 and Pol IVb: a versatile pathway for transcriptional gene silencing in plants. **Biochim Biophys Acta** 1769: 358-374.

*Matzke M, Kanno T, Huettel B, Daxinger L, Matzke AJM (2007a) Targets of RNA-directed DNA methylation. **Curr Opin Plant Biol** 10: 512-519.

Tops, B.B., Plasterk, R.H.A. and Ketting, R.F. (2007), C. elegans Argonaute proteins ALG-1 and ALG-2; almost identical yet different. **Cold Spring Harb Symp Quant Biol**. 71:189-94

*Matzke M, Kanno T, Huettel B, Daxinger L, Matzke AJM (2007b) RNA-directed DNA methylation and Pol IVb in Arabidopsis. **Cold Spring Harb Symp Quant Biol**. 71: 449-459.

Ketting RF (2007) A Dead End for MicroRNAs.**Cell**.131(7):1226-7.

Presentations in Scientific Meetings

- Oral presentations (indicate invited / keynote talks)

Invited talks Ketting:

1. Germ Cell Meeting, Cold Spring Harbor, USA (October 2006)

2. miRNA Europe, Cambridge, England (Nov 2006)

3. Stem Cells 2006, Cancun, Mexico (Dec 2006)

4. Microsymposium on small RNAs, Vienna, Austria (May 2007)

5. RNAi Copenhagen Symposium, Copenhagen, Denmark (May 2007)

6. Linnaeus Workshop on New Mechanisms in the Evolution of Phenotypic Diversity, Uppsala, Sweden (Oct. 2007)

7. RNAi2008, Oxford, England (March 2008) (Keynote lecture)

8. Keystone symposium on RNAi, MicroRNA and Non-Coding RNA, Whistler, Canada (March 2008)

Invited talks Matzke:

1. 71st Cold Spring Harbor Symposium on Regulatory RNAs, Cold Spring

Harbor, June 2006

2. International Society of Plant Molecular Biology, Keynote speaker,

Adelaide, Australia, Aug. 2006

3. 24th UC-Riverside symposium in plant biology (Gene silencing: the

biology of small RNAs and the epigenome), California, USA, Jan 2007

4. Keystone conference on miRNAs and siRNAs: biological functions and

mechanisms, Colorado, USA, Jan. 2007

5. Plant Regulatory RNAs, Taipei, Taiwan, March 2007

6. International Conference on polyploidy, heterosis and epigenetics,

Beijing, May 2007

7. Systems Biology Workshop: from nucleotide to ecosystem, Melbourne, Australia, May 2007

8. 32nd Annual FEBS Conference, Symposium speaker and session chair,

Vienna, July 2007

9. Gordon Research Conference on Epigenetics, New Hampshire, USA, Aug. 2007

10. Lorne Genome Conference, Australia, Feb. 2008

11. Keystone conference on RNAi, miRNAs, noncoding RNAs, Canada, March 2008

Talks Kanno:

1. Molecular analysis of Rina-directed DNA methylation and transcriptional gene silencing in Arabidopsis (Invited speaker), The International Conference “Plant Transformation Technologies” ; Vienna, Austria, (Feb 2007)

2. Genetic analysis of RNA-directed DNA methylation pathway in Arabidopsis thaliana (Invited speaker), 4th Tri-National Arabidopsis Meeting; Vienna, Austria (Sept 2007)

3. An SMC hinge domain-containing protein is required for RNA-directed DNA methylation (oral presentation, seminar series at Gregor Mendel Institute), Vienna, Austria (Dec 2007)

Talks Kamminga:

1. EuroDYNA meeting, Brno, Czech Republic (Oct 2006)

2. Nucleic Acids Meeting, Lunteren, Netherlands (Dec 2007)

- Posters

-Keystone symposium on miRNAs and siRNAs, Keysone, USA (January 2007)

-Keystone symposium on RNAi, MicroRNA and Non-Coding RNA, Whistler, Canada (March 2008)

-Targets and functions of RNA-directed DNA methylation and

Pol IVb in Arabidopsis thaliana (poster), Brno, Czech Republic (Oct 2006)

-RNA2006 Meeting, Seattle, USA (June 2006)

Public Outreach:

- Press releases

- National / international Newspaper articles (presenting your CRP or part of your work)

- TV appearance

- Radio appearance

- Other (*please define*)

Other Activities / Products

- Patents

- Websites

- Other (*please define*)

IMPORTANT: In your list, please identify with an asterisk (*) those publications and contributions in which the EuroDYNA Programme is properly acknowledged.

Control of stress and interferon regulated gene expression by transcription factors, histone modification and nuclear compartmentalisation

Abstract

The precise regulation of gene expression in response to extracellular stimuli plays a key role in life and biological diversity. Specific transcription factors, general transcription machinery, histone modifying enzymes, chromatin remodeling complexes, as well as nuclear architecture all have roles in gene transcription. How these individual events are coordinated in time and space, and integrated into appropriate transcriptional responses is a challenging yet unresolved question. We will address this issue using as a model the transcription of stress and interferon regulated genes. Both signalling pathways can be activated by independent stimuli and may therefore be studied separately. However, under physiological conditions, the stress and interferon signalling cascades are often activated simultaneously resulting in enhanced transcriptional responses. This synergism is essential for defense against pathogens and in tumor surveillance. Our studies on the order, location and contribution of stress and interferon-induced changes in chromatin modification and nuclear architecture will improve our understanding of the regulation of gene expression and open up new possibilities to combat diseases, such as cancer and infections. The availability of cells and animals with specific genes of the stress and interferon pathways inactivated will enable us to test the working hypotheses under physiological conditions.

Partners

(FWF, GAČR)

Dr Pavel Kovarik (Project Leader)
Institute of Microbiology and Genetics, Vienna, Austria

Dr Pavel Hozák
Institute of Molecular Genetics, Prague, Czech Republic

Part A. Collaborative Research Project (CRP)

→ (To be completed by Project Leader)

A.1. General information about the Collaborative Research Project (CRP)
CRP Number: 03-DYNA–F-20 **CRP Title and Acronym**: **Control of Stress and Interferon regulated Gene Expression by Transcription Factors, Histone Modification and Nuclear Compartmentalisation / Stress- and Interferon-induced Transcription and Nuclear Reorganization** **Name Project Leader**: Dr. Pavel Kovarik **Project-related website**: http://www.univie.ac.at/eurodyna/ **Reporting period**: from 01/01/2005 to 01/01/2008 (Please report for the whole duration of your project)
List all Principal Investigators (PIs), Associated Partners (APs) and Co-operating Partners (CPs) of the CRP
PI 1
name: **Pavel Kovarik**
PI 2
name: **Pavel Hozak**

A.3. Report of the CRP in terms of Networking, Training and Dissemination

→ (To be completed by Project Leader with input from CRP members)

Please provide a concise and clear description of the networking, training and dissemination activities undertaken by this CRP, addressing the following issues:

6. Networking activities: Of all the networking activities developed during this period, please highlight the most important one for this CRP (in terms of outcome, impact, opportunities for trans-national collaborations and synergy with other European and international initiatives)

1) Networking with 03-DYNA-F-18 "Spatio-temporal organisation of genome surveillance in live cells": Exchange of reagents and expertise, short (up to several weeks) visits to the lab of Dr. Bartek. As a result, a joint research grant has been approved.

2) Networking with 03-DYNA-F-03 "Cell biology of messenger RNA biogenesis": Exchange of reagents and expertise, a 2 week training visit of a PhD student to the lab of Dr. Fonseca. The technology transfer was essential for a grant proposal (decision due in May).

3) Networking with 03-DYNA-F-32 "Environmental stress-induced dynamic modulation of chromatin structure, gene expression and nuclear architecture in yeast": Exchange of information on p38- and Hog1-dependent chromatin-relevant phosphorylation events in mammalian and yeast cells, respectively. Two meetings in addition to the annual Eurodyna conferences were organized.

4) Networking with QUASI (EU FP6 project): Two joint meetings on "Chromatin-associated phosphorylation and dephosphorylation events" were organized. A joint grant proposal for FP7 is being evaluated.

7. Training activities: Please, highlight the most useful training activity (workshop, course or school) undertaken by senior or junior researchers of this CRP

PK has coordinated a successful application for a doctoral school for 10 PhD students funded by the University of Vienna on the Eurodyna-related topic "Functional organization of the nucleus" (http://www.univie.ac.at/ik-cellnucleus/). Although only shortly (approx. 1 year) overlapping with the funding period of Eurodyna the doctoral school will profit from the Eurodyna network through the established links and maintaining them in the future.

8. Dissemination activities: What has this CRP done to promote the EuroDYNA Programme more actively and widely? Please, indicate the outcome and impact of the dissemination activities developed during this period

1) PK has co-organized the Eurodyna 2007 conference in Brno.

2) Eurodyna was presented to the University of Vienna as a valuable network for a better training of PhD students in the doctoral school funded by the University of Vienna.

3) Networking with QUASI (EU FP6 project)

A.4 Valuable feedback from this CRP

→ (To be completed by Project Leader with input from CRP members)

Please, provide a <u>concise and clear answer</u> to these questions:

9. Key contribution of this CRP to the EURODYNA Programme: How has the EuroDYNA Programme benefited from this CRP? Please, provide one example that clearly illustrates your valuable input (whether scientific, networking, training and/or dissemination input)

1) First description of a chromatin-associated phosphorylation of a transcription factor in mammalian cells (favorable revision in PNAS). This finding is likely to launch efforts to characterize similar events linked to other transcription factors than Stats. Such events will shed new light into regulatory switches during transcription.

2) Role of interferons and Jak/Stat signaling in genome surveillance and DNA-damage response.

10. Key contribution of the EURODYNA Programme to this CRP: How has your participation in the EuroDYNA Programme influenced your research (in terms of visibility, collaborations, opportunities, ideas)

In general, Eurodyna enabled the two participating labs in this CRP to establish new links and enter novel research field. In the case of this CRP the Eurodyna environment was particularly important since the CRP was severely hampered by absence of the two favorably evaluated colleagues from the UK. The situation was further complicated by the unexpected data on PML and PML nuclear bodies.

Specifically:

1) Dr. Hodny from the P. Hozak´s lab became an independent scientist. Eurodyna certainly contributed to this step.

2) PK was able to increase and stabilize the number of the lab members to 5 which allows a sustainable

development of the group.

3) Both groups entered new research fields: P. Hozak and his coworker Z. Hodny established DNA-damage and senescence research in their labs; P. Kovarik has initiated a project on RNA stability and its links to transcription.

4) The CRP profited greatly from the Eurodyna-funded short-term fellowships for visits of students to other Eurodyna labs. This was indeed a great instrument since the underlying procedure was informal with a very little paper work.

5) The Eurodyna network helped in the successful application for the doctoral school at the University of Vienna.

A.5 Follow up related to the CRP and EuroDYNA Programme

→ (To be completed by Project Leader with input from CRP members)

11. Follow up: Please provided details of the most important new initiatives (either within a national or an international context) that have been developed as a result of the collaboration of this CRP and the EuroDYNA Programme.

1) As mentioned above: Doctoral school ““Functional organization of the nucleus” (http://www.univie.ac.at/ik-cellnucleus/), University of Vienna.

2) EU project on chromatin-associated kinases in preparation

A. 5. List of Publications, Dissemination and Outreach Activities undertaken by the CRP

→ (To be completed by Project Leader with input from CRP members)

Publications

- Articles

Peer reviewed articles in journals (published, in press or submitted)

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Sauer I., Schaljo B., Vogl C., Gattermeier I., Kolbe T., Muller M., Blackshear P.J., and Kovarik P. 2006. Interferons limit inflammatory responses by induction of tristetraprolin. Blood, 107:4790-7

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Vlasakova, J., Novakova, Z., Rossmeislova, L., Kahle, M., Hozak, P. and Hodny, Z. (2007) Histone deacetylase inhibitors suppress IFN{alpha}-induced up-regulation of promyelocytic leukemia protein. Blood, 109, 1373-1380.

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Janderova-Rossmeislova, L., Novakova, Z., Vlasakova, J., Philimonenko, V., Hozak, P. and Hodny, Z. (2007) PML protein association with specific nucleolar structures differs in normal, tumor and senescent human cells. Journal of Structural Biology, 159, 56-70.

Piskacek, S., Gregor, M., Nemethova, M., Grabner, M., Kovarik, P., and Piskacek, M. (2007) Nine-amino-acid transactivation domain: Establishment and prediction utilities. Genomics, 89:756-768.

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Novakova, Z., Janderova-Rossmeislova, L., Vlasakova, J., Vasicova, P., Horejsi, Z., Bartek, J., Hozak, P. and Hodny, Z. Sustained activation of JAK/STAT signalling pathway and induction of interferon stimulated genes in 5-bromo-2'-deoxyuridine and distamycin A-induced senescence. Aging Cell, *submitted*.

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Kovarik P, Sauer I, Schaljo B. 2008 Molecular mechanisms of the anti-inflammatory functions of interferons. Immunobiology. 212(9-10):895-901.

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Iwona Sadzak, Melanie Schiff, Irene Gattermeier, Reingard Glinitzer, Ines Sauer, Armin Saalmüller, Edward Yang, Barbara Schaljo and Pavel Kovarik¶ . Recruitment of Stat1 to chromatin is required for interferon-induced serine phosphorylation of Stat1 transactivation domain. *Under favorable revision in PNAS*

Presentations in Scientific Meetings

- Oral presentations (indicate invited / keynote talks)

Selected oral presentation:

48th Symposium of the Society for Histochemistry, Stresa, Italy, 7.9.-10.9.2006

PML protein association with specific nucleolar structures differ in normal, tumor and senescent human cells (oral presentation, Rossmeislova L.)

Selected oral presentation:

Ines Sauer, Barbara Schaljo, Pavel Kovarik. Interferons limit inflammatory responses by induction of Tristetraprolin. 6th International Cytokine Conference 2006, August 27-31, Hilton-Stadtpark, Vienna, Austria

Invited talk:

Pavel Kovarik, Ines Sauer, Barbara Schaljo. Role of interferons in the decline of inflammation. 20th Annual Conference of the European Macrophage & Dendritic Cell Society (EMDS); FREIBURG, Germany October 5th to 7th, 2006

Short talk:

Melanie Schiff., Irene Gattermeier, Reingard Glinitzer, Iwona Sadzak, Ines Sauer, Barbara Schaljo and Pavel Kovarik. 2007 RECRUITMENT OF STAT1 TO CHROMATIN IS REQUIRED FOR INTERFERON-INDUCED SERINE 727 PHOSPHORYLATION. Jaks, Stats and Immunity. Keystone meeting, Jan 5-10, 2007

Selected oral presentation:

Z. Novakova, L. Janderova-Rossmeislova, J. Vlasakova, P. Hozak and Z. Hodny: Activation of JAK/STAT signalling pathway and induction of interferon stimulated genes in BrdU-induced senescent cells, 20th Wilhelm Bernhard Workshop: International Conference on the Cell Nucleus, St Andrews, UK, 27. – 31. 8. 2007

- Posters

Dynamic Organization of Nuclear Function, Cold Spring Harbor Laboratory, USA, 27. 9. - 5. 10.2006

Histone deacetylase inhibitors suppress IFNα-induced up-regulation of PML (poster presentation, Vlasakova J.)

48th Symposium of the Society for Histochemistry, Stresa, Italy, 7.9.-10.9.2006

The Role of PML in 5-BrdU-induced senescence (poster presentation, Novakova Z.)

Molecular Genetics of Aging, Cold Spring Harbor, USA, 2.10.-9.10.2006

Molecular Aspects of Cellular Senescence Induced by 5-Bromodeoxyuridine in Vitro (poster presentation, Novakova Z.)

Barbara Schaljo, Iwona Sadzak, Ines Sauer and Pavel Kovarik. Synergy of p38MAPK and Stat1 in transcription. Cell Signaling, January 25th to January 28th 2006, Luxembourg (Poster presentation)

Barbara Schaljo, Iwona Sadzak, Franz Kratochvill, Pavel Kovarik. STAT1 TARGETS p38 MAPK-MEDIATED CHANGES IN GENERAL TRANSCRIPTION COMPLEXES TO SPECIFIC PROMOTERS. EuroDyna Meeting, Mendel Center, Brno - 12 – 14 October 2006 (Poster presentation)

Iwona Sadzak. EMBO Workshop: Functional Organization of the Cell Nucleus, Prague May 5 – 8, 2006

Melanie Schiff, Irene Gattermeier, Reingard Glinitzer, Barbara Schaljo, Ines Sauer, Wilhelm Gerner, Armin Saalmüller and Pavel Kovarik. Nuclear import and prephosphorylation control interferon-induced Stat1 serine phosphorylation. 6th International Cytokine Conference 2006, August 27-31, Hilton-Stadtpark, Vienna, Austria (Poster presentation)

Z. Nováková, L. Rossmeislová, J. Dobrovolná, S. Hubáčková, P. Hozák and Z. Hodný: The role of JAK/STAT signaling pathway in cellular senescence (poster), Inflammation and Cancer – Lecture Course and International Workshop, Milan, Italy, 7. – 9. 11. 2007

Josephine Grass, Barbara Schaljo-Kapp and Pavel Kovarik (2007) Role of Cdk9 in Stat1–dependent transcription. The 32nd FEBS congress 2007 will take place from July 7 - 12, 2007 at the Congress Center MessezentrumWienNeu (New Vienna fairgrounds), A-1021 Wien, Messeplatz

Iwona Sadzak and Pavel Kovarik, BIOGENESIS OF STAT SIGNALING COMPLEXES, ELSO 2007, 1-4 September 2007 in Dresden, Germany

Josephine Grass, Barbara Schaljo-Kapp and Pavel Kovarik (2007) Role of Cdk9 in Stat1–dependent transcription. ELSO 2007, 1-4 September 2007 in Dresden, Germany

Public Outreach:

- Press releases

- National / international Newspaper articles (presenting your CRP or part of your work)

- TV appearance

- Radio appearance

Other Activities / Products

- Patents

- Websites

IMPORTANT: In your list, please identify with an asterisk (*) those publications and contributions in which the EuroDYNA Programme is properly acknowledged.

The role of linker histone variants and their phosphorylation in chromatin structure and function

Abstract

The linker histones are known to contribute to the formation and maintenance of higher order chromatin structures but their physiological functions are still largely unknown. They display a complex pattern of variants and recent data suggest that they may have specific roles in epigenetic control of gene expression. The cell cycle dependent phosphorylation of certain serine and threonine residues in the charged tails of the linker histones is most probably of major importance in determining the architecture of chromatin during cell proliferation and differentiation, but the molecular details of this process are very unclear. Aberrant chromatin structure may contribute to malignant transformation and tumour formation. This project aims at elucidating these mechanisms by combining the expertise of Herbert Lindner's laboratory in Innsbruck and Jean Thomas' laboratory in Cambridge, in collaboration with Ingemar Rundquist's laboratory in Linköping. All three research groups have many years' experience in linker histone research and the laboratories complement each other well, offering a large range of methods and techniques in analytical chemistry, structural biology, biophysics, and cytochemistry. Together, these investigations should contribute to increased understanding of epigenetic mechanisms involved in chromatin architecture, regulation of cell growth and differentiation, and in malignant transformation and tumour progression.

Partners

(FWF, MRC)

Prof Herbert Lindner (Project Leader)
University of Innsbruck, Austria

Prof Jean O. Thomas
University of Cambridge, UK

Associated Partner:

Prof Ingemar Rundquist
University of Linköping, Sweden

Part A. Collaborative Research Project (CRP)

→ (To be completed by Project Leader)

A.1. General information about the Collaborative Research Project (CRP)
CRP Number: 03-DYNA–F-23 **CRP Title and Acronym**: **The Role of Linker Histone Variants and their Phosphorylation in Chromatin Structure and Function / H1 Phosphorylation** **Name Project Leader**: Prof. Herbert Lindner **Project-related website**: **Reporting period**: from 01/04/2005 to 01/04/2008 (Please report for the whole duration of your project)
List all Principal Investigators (PIs), Associated Partners (APs) and Co-operating Partners (CPs) of the CRP
PI 1
name: **Prof. Herbert Lindner**
PI 2
name: **Prof. Jean O. Thomas**
AP 1
name: **Prof. Ingemar Rundquist**

A.3. Report of the CRP in terms of Networking, Training and Dissemination

→ (To be completed by Project Leader with input from CRP members)

Please provide a <u>concise and clear</u> description of the networking, training and dissemination activities undertaken by this CRP, addressing the following issues:

6. Networking activities: Of all the networking activities developed during this period, please highlight the most important one for this CRP (in terms of outcome, impact, opportunities for trans-national collaborations and synergy with other European and international initiatives)

Due to the outcome of our CRP, we were able to establish collaboration with other European research groups working in the field of linker histones. This initiation of gathering relevant European research groups in this field could be of importance for the progress in this complex research area.

7. Training activities: Please, highlight the most useful training activity (workshop, course or school) undertaken by senior or junior researchers of this CRP

One PhD student from Linköping (Elisavet Koutzamani) received a substantial part of her training in the Innsbruck laboratory.

The PhD student associated with the project in Cambridge (Laura Cato) received valuable training in NMR spectroscopy of linker histones alongside her normal biochemical training; this would not have occurred in the absence of the EuroDyna project. The same applies to the EuroDyna postdoc who had no previous NMR experience.

8. Dissemination activities: What has this CRP done to promote the EuroDYNA Programme more actively and widely? Please, indicate the outcome and impact of the dissemination activities developed during this period

Modified and unmodified histone micro sequence variants have been supplied by the Innsbruck lab to other CRP's ,but it was not possible to establish further collaborations within the EuroDYNA programme.

A.4 Valuable feedback from this CRP

→ (To be completed by Project Leader with input from CRP members)

Please, provide a concise and clear answer to these questions:

9. Key contribution of this CRP to the EURODYNA Programme: How has the EuroDYNA Programme benefited from this CRP? Please, provide one example that clearly illustrates your valuable input (whether scientific, networking, training and/or dissemination input)

The Innsbruck/Cambridge/Linkoping CRP lies at the heart of the EuroDyna programme, as summarised on the website, and addresses fundamental aspects of chromatin structure. A key step in the control of gene expression is the transition between the 'open' and 'closed' states of chromatin, reflecting differences in packing. Linker histones have a key role in this process and this role is modulated by phosphorylation. We seek to characterise and understand the range of phosphorylation states (e.g. during the cell cycle) using specialized analytical methods such as HILIC, and at a molecular level (using NMR and other biophysical techniques) the effect of phosphorylation on linker histone structure and on histone/DNA and histone/chromatin interactions. We are the only CRP working at this most fundamental level, and this is probably our key contribution as a CRP to the EuroDyna Programme.

10. Key contribution of the EURODYNA Programme to this CRP: How has your participation in the EuroDYNA Programme influenced your research (in terms of visibility, collaborations, opportunities, ideas)

The EURODYNA Programme has been the catalyst of extensive collaboration and exchange of ideas between our research groups.

A.5 Follow up related to the CRP and EuroDYNA Programme

→ (To be completed by Project Leader with input from CRP members)

11. Follow up: Please provided details of the most important new initiatives (either within a national or an international context) that have been developed as a result of the collaboration of this CRP and the EuroDYNA Programme.

see point 6

A. 5. List of Publications, Dissemination and Outreach Activities undertaken by the CRP

→ (To be completed by Project Leader with input from CRP members)

Publications

- Articles

Peer reviewed articles in journals (published, in press or submitted)

Lindner HH: Analysis of histones, histone variants, and their posttranslationally modified forms.

Electrophoresis 2008 (in press)

Rundquist I. and Lindner H.H.: Analyses of linker histone – chromatin interactions in situ. Biochem. Cell Biol. 84:427-436, 2006

Sarg B, Helliger W, Talasz H, Förg B, and Lindner HH: Histone H1 phosphorylation occurs site-specifically during interphase and mitosis. Identification of a novel phosphorylation site on histone H1. J.Biol.Chem. 281,6573-6580,2006

Sarg B, Green A, Soderkvist P, Helliger W, Rundquist I, and Lindner HH: Characterization of sequence variations in human histone H1.2 and H1.4 subtypes. FEBS J. 272, 3673-3683 (2005)

Gréen A, Sarg B, Genheden U, Koutzamani E, Lindner HH & Rundquist I (2008) Histone H1 dephosphorylation is not a general feature in early apoptosis. *Second revision submitted to Biochemistry*.

Kostova NN, Srebreva L, Markov DV, Sarg B, Michael RA, Thomas JO, Lindner HH & Rundquist I Histone H5 - chromatin interactions in situ are strongly modulated by C-terminal phosphorylation. *Manuscript, almost ready for submission*

B. Sarg, C. Chwatal, H. Talasz and HH. Lindner: Testis specific linker histone H1t is multiple phosphorylated during spermatogenesis: Identification of the phosphorylation sites *Manuscript, almost ready for submission*.

L. Cato, K. Stott, M Watson and J.O. Thomas: The interaction of HMGB1 with linker histones H1 and H5 occurs through its acidic tail and is stable at 'physiological' ionic strength. (Provisional title; manuscript almost ready for submission).

- Books

As editor(s)

Koutzamani E.: Chromatin, Histones and Epigenetic Tags. PhD thesis, Linköping University Medical Dissertations No. 960, 2006. ISBN 91-85523-10-0. http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-7687

As author(s)

*Sarg B and Lindner H: Capillary electrophoresis of post-translationally modified proteins and peptides: Handbook of Capillary and Microchip Electrophoresis and Associated Microtechniques,Third Edition edited by James P. Landers (2008)

Presentations in Scientific Meetings

- Oral presentations (indicate invited / keynote talks)

*Herbert H. Lindner: Powerful Analytical Techniques for the Separation of Phosphorylated Proteins

Max Plank Institute für Immunbiologie, 28th August 2007, Freiburg, Germany (invited)

*Herbert H. Lindner: New and Efficient Methods for the Analysis of Linker Histones and their Posttranslational Modifications (keynote)

Symposium: “Histone H1: Why so many isoforms?”, 15th September 2005, Barcelona, Spain (invited)

*Bettina Sarg: Edman Degradation and Mass Spectrometry (MS/MS) for the Determination of Posttranslational Histone Modifications (invited)

Symposium: “Histone H1: Why so many isoforms?”, 15th September 2005, Barcelona, Spain (invited)

Herbert Lindner:The role of linker histone variants and their phosporylation in chromatin structure and function

EURODYNA Conference 2005, 22.-24. 9. 2005, Thun, Switzerland

Jean Thomas : Chromatin structure, linker histones and HMG-box proteins Structural Genomics Consortium Symposium, 8 March 2006, Karolinska Institute. Stockholm, Sweden (Invited)

Jean Thomas: Linker histones and their interactions with DNA and chromatin

EURODYNA Conference 2006, 12.-16 October 2006, Brno, Czech Republic

Jean Thomas : Chromatin structure, linker histones and HMG-box proteins. NACON international conference, April 2007, Sheffield, UK (invited ; plenary)

- Posters

*Rundquist I. and Lindner H.: Analyses of linker histone – chromatin interactions in situ in different cell systems. 2:nd Chromatin Structure and Function Meeting, Nassau, Bahamas 15-18 November, 2005

*Nora Kostova, Bettina Sarg, Herbert Lindner, and Ingemar Rundquist

Analyses of linker histone – chromatin interactions *in situ*

EURODYNA Conference 2006, 12.-16.10.2006, Brno, Czech Republic

*Bettina Sarg, Wilfried Helliger, Heribert Talasz, Barbara Förg, Jean Thomas and Herbert Lindner: Histone H1 phosphorylation occurs site-specifically during interphase and mitosis.

EURODYNA Conference 2006, 12.-16.10.2006, Brno, Czech Republic

*Bettina Sarg, Heribert Talasz, Barbara Förg, Wilfried Helliger and Herbert Lindner: Analysis of Histone H1 Phosphorylation During Interphase and Mitosis by HPCE.
LACE 2006, 2.-5-12.2006, Queretaro, Mexico

Public Outreach:

- Press releases

- National / international Newspaper articles (presenting your CRP or part of your work)

- TV appearance

- Radio appearance

- Other (*please define*)

Jean Thomas: two public lectures (open to non-scientists and scientists);

• Cambridge Philosophical Society evening lecture, 'Packaging the genome: chromatin. DNA architecture and the role of proteins". 28 November 2005, University of Cambridge, UK

•Amalgamated Societies Lecture (evening), "DNA, chromatin and chromosomes". 29 Janiary 2008, St Catharine's College, University of Cambridge, UK

Other Activities / Products

- Patents

- Websites

- Other (*please define*)

IMPORTANT: In your list, please identify with an asterisk (*) those publications and contributions in which the EuroDYNA Programme is properly acknowledged.

Chromatin higher order dynamics: a single molecule approach

Abstract

Higher order structure of eukaryotic chromosomes is governed by protein/DNA interactions that mediate the folding of DNA into chromatin fibres. Chromatin fibre structure revolves around nucleosomes, the fundamental units of chromatin. SNF2 ATPases and histone modifying enzymes remodel nucleosomes and have been documented to play key roles in the generation, maintenance and alteration of the epigenetic code during the cell cycle and during ontogeny. We propose to study the influence of chromatin remodelling factors on the physical properties of chromatin fibres. To this end, fully recombinant model polynucleosomal arrays suited for physico-chemical characterisation will be generated. Second, defined prototypic chromatin remodelling activities will be purified in preparative quantities. Last, state-of-the-art single molecule magnetic tweezers and time-lapse Atomic Force Microscopy will be employed to rigorously investigate the physical properties and dynamics of chromatin higher order structural transitions catalysed by chromatin remodellers. With this combined multidisciplinary approach we expect to elucidate nucleosome mediated higher order chromatin structural transitions with an unsurpassed degree of resolution.

Partners

(NWO, DFG)

Dr Colin Logie (Project Leader)
University of Nijmegen, The Netherlands

Dr Alexander Brehm
Philipps-Universität Marburg, Germany

Dr. John van Noort
University of Leiden, The Netherlands

Part A. Collaborative Research Project (CRP)

→ (To be completed by Project Leader)

A.1. General information about the Collaborative Research Project (CRP)
CRP Number: 03-DYNA–F-25 **CRP Title and Acronym**: **Chromatin Higher Order dynamics: A Single Molecule Approach** **Name Project Leader**: Dr. Colin Logie **Project-related website**: **Reporting period**: from 01/09/2005 to 01/09/2008 (Please report for the whole duration of your project)
List all Principal Investigators (PIs), Associated Partners (APs) and Co-operating Partners (CPs) of the CRP
PI 1
name: **Colin Logie**
PI 2
name: **Alexander Brehm**
PI 3
name: **John van Noort**

A.3. Report of the CRP in terms of Networking, Training and Dissemination

→ (To be completed by Project Leader with input from CRP members)

Please provide a <u>concise and clear</u> description of the networking, training and dissemination activities undertaken by this CRP, addressing the following issues:

6. Networking activities: Of all the networking activities developed during this period, please highlight the most important one for this CRP (in terms of outcome, impact, opportunities for trans-national collaborations and synergy with other European and international initiatives)

This involved the physicist John van Noort visiting the MRC in Cambridge and setting–up a very fruitfull collaboration with Daniela Rhodes.

7. Training activities: Please, highlight the most useful training activity (workshop, course or school) undertaken by senior or junior researchers of this CRP

The workshop in Corsica, organized by John van Noort, to which Joke van Vugt participated.

8. Dissemination activities: What has this CRP done to promote the EuroDYNA Programme more actively and widely? Please, indicate the outcome and impact of the dissemination activities developed during this period

Organization of a EuroDYNA –sponsored subgroup meeting entitled 'Chromatin and the cell cycle' at the ELSO 2007 conference in Dresden. See also the Pod-cast at http://www.esf.org/media-centre/podcast-and-video-gallery/podcast-from-seppia.html for a further PR activity linked to the ELSO subgroup meeting.

A.4 Valuable feedback from this CRP

→ (To be completed by Project Leader with input from CRP members)

Please, provide a <u>concise and clear answer</u> to these questions:

9. Key contribution of this CRP to the EURODYNA Programme: How has the EuroDYNA Programme benefited from this CRP? Please, provide one example that clearly illustrates your valuable input (whether scientific, networking, training and/or dissemination input)

The Corsica summer school brought 10 EuroDYNA lab members together and also allowed them to meet potential collaborators as well as their concollegae.

10. Key contribution of the EURODYNA Programme to this CRP: How has your participation in the EuroDYNA Programme influenced your research (in terms of visibility, collaborations, opportunities, ideas)

The effect of obtaining this grant has been galvanizing, it has spurred us to take on the organization of several activities (Summer School, ELSO chromatin session) as well as lending us international credibility in the chromatin biophysics field. One further interesting development involves Colin Logie and Karen Edler from the ESF programme 'Self-Organizing Nano Structures' who are applying for grants and discussing nanotechnology alternatives to what is becoming known as 'synthetic biology'. This latter development is largely due to the drive of the ESF office who organized SONS-EuroDYNA meetings in Brussels and in Lisbon in September 2006 and December 2007.

A.5 Follow up related to the CRP and EuroDYNA Programme

→ (To be completed by Project Leader with input from CRP members)

11. Follow up: Please provided details of the most important new initiatives (either within a national or an international context) that have been developed as a result of the collaboration of this CRP and the EuroDYNA Programme.

Application for grants called by the ERC NanoSci-E+ on interfacing nanocompartments by principal investigators Edler, Cavalli-Petraglia, Magner, Logie and Onida

A. 5. List of Publications, Dissemination and Outreach Activities undertaken by the CRP

→ (To be completed by Project Leader with input from CRP members)

Publications

- Articles

Peer reviewed articles in journals (published, in press or submitted)

Murawska M, Kunert N, van Vugt J, Längst G, Kremmer E, Logie C, Brehm A. dCHD3, a novel ATP-dependent chromatin remodeler associated with sites of active transcription. Mol Cell Biol. (2008) 8:2745-57. *****

Kruithof M, Chien F, de Jager M, van Noort J. Subpiconewton dynamic force spectroscopy using magnetic tweezers. Biophys J. (2008) 94(6):2343-8.

Koopmans WJ, Brehm A, Logie C, Schmidt T, van Noort J. Single-pair FRET microscopy reveals mononucleosome dynamics. J Fluoresc. (2007) 6:785-95. *****

Campsteijn C, Collin-Wijnands AMJ, Logie C. Reverse Genetic Analysis of the Yeast RSC Chromatin Remodeler Reveals a Role for RSC3 and SNF5 Homolog 1 in Ploidy Maintenance. PLoS Genet. (2007) 3(6): e92

van Vugt JJ, Ranes M, Campsteijn C, Logie C. The ins and outs of ATP-dependent chromatin remodeling in budding yeast: biophysical and proteomic perspectives. Biochim Biophys Acta. (2007) 1769(3):153-71.*****

Bouazoune K. and Brehm A. ATP-dependent chromatin remodeling complexes in Drosophila Chromosome Res. (2006), 14, 433-449.

Bouazoune K. and Brehm A. dMi-2 chromatin binding and remodeling activities are regulated by dCK2 phosphorylation. J. Biol. Chem. (2005), 280, 41912-41920.

Ozdemir A, Masumoto H, Fitzjohn P, Verreault A, Logie C. Histone H3 lysine 56 acetylation: a new twist in the chromosome cycle. Cell Cycle. (2006) 5(22):2602-8. *****

Engeholm M, de Jager M, Flaus A, van Noort J and Owen-Hughes T. Nucleosomes are not excluded from DNA territories occupied by their neighbours, submitted.*****

Kruithof M, Chien F, Routh A, Logie C, Rhodes D, van Noort J Single-Molecule Force Spectroscopy

Reveals a Highly Compliant Solenoidal Structure for the 30 nm Chromatin Fiber, submitted. **

van Vugt J., de Jager M., Brehm A., van Noort J. and Logie C. Determination of the nucleosomal stepsize induced by ATP-dependent chromatin remodelling enzymes, submitted. **

- Books

As editor(s)

As author(s)

Kunert N. and Brehm A., Mass production of Drosophila embryos and chromatographic purification of native protein complexes.

(Book chapter in "Drosophila" by Humana Press; (2007)

M. de Jager & S.J.T. van Noort: Atomic force microscopy. Encyclopedia of Life Sciences (2007) 1-10, eds. John Wiley & Sons, Ltd.

Presentations in Scientific Meetings

- Oral presentations (indicate invited / keynote talks)

Colin Logie

ESF-EuroDYNA conference: Thun, Switzerland, September 2005 – invited speaker. **

Delft Technical university, Biolunch seminars, The Netherlands, February 2006 – invited speaker. **

ESF- EMBO sysmposium 'Gene transcription in yeast' : Barcelona, Spain, June 2006 – invited speaker. **

Telomeres and genome stability EMBO conference, Villars sur Ollon, September 2006 – selected speaker. *

ESF-EuroDYNA conference: Brno, Oct 2006 – invited speaker. **

Manchester University seminar, Manchester, November 2006 – invited speaker. **

Chromosome dynamics, NCMLS forum Nijmegen, February 2007 – invited speaker.

Chromatin remodelling, Marburg, Germany, April 2007 – invited speaker. **

Chromatin and the cell cycle. European Life Sciences Organisation (ELSO) bi-annual meeting Dresden,

Germany, Septembre 2007 –invited speaker.*****

High-resolution proteomic analysis of the SWI/SNF-related S. cerevisiae RSC ATP-dependent chromatin remodeling complex. National cancer Institute (NKI), Dutch chromatin meeting, Amsterdam, October 2007 – selected speaker. *****

Determination of the nucleosomal step size of RSC and Mi2. Gordon Research Conference on Chromatin Structure and Function, Italy, May 2008 –selected speaker. *****

Alexander Brehm

10th Japanese-German Workshop on Molecular and Cellular Aspects of Carcinogenesis: Essen, Germany, October 2005 – invited speaker. *****

Seminar series on "Molecular Oncology and Immunology": Göttingen, Germany, March 2006 – invited speaker. *****

19th EACR Meeting: Budapest, Hungary, July 2006 – invited speaker. *****

Workshop "Protein-Nucleic Acid Interactions" of the International Research Training Group Gießen/Marburg-Moscow (GRK1384), Suzdal, June 2007 – invited speaker.

Magdalena Murawska (Brehm group)

Workshop "Protein-Protein Acid Interactions" of the International Research Training Group Gießen/Marburg-Moscow (GRK1384), Gießen, March 2008 – invited speaker.

John van Noort:

- Single-molecule dynamics of chromatin remodeling; getting around nucleosomes. MRC, Laboratory of Molecular Biology, Cambridge, U.K., 14 June 2005.

- Single-molecule biopohysics of epigenetic regulation.

  Philips Natuurkundig Laboratorium, Eindhoven, The Netherlands, 26 July 2005.

- Chromatin higher order dynamics: a single molecule approach.

  First EuroDYNA Conference, Thun, Switzerland, 22-24 September 2005, oral. *****

- Chromatin dynamics: a single molecule approach. *****

  Complex Fluids and Biophysics Seminar, Instituut Lorentz, Leiden, The Netherlands, 1 November 2005.

- Chromatin dynamics: a single molecule approach. *****

Josephine Nefkens Instituut, Erasmus University, Rotterdam, The Netherlands, 11 November 2005.

- Single-molecule experiments on chromatin dynamics. Chromatin Workgroup III, Rolduc Conference Center, Kerkrade, The Netherlands, 7-8 December 2005.

- Physical techniques to uncover the physics of chromatin; single molecule studies. *****

  ESF Brainstorm Session SONS/DYNA, Brussels, Belgium, 27 September 2006.*****

- Uncovering – physical mechanisms of – epigenetic control. Epigenetics meeting, LUMC, Leiden, 5 October 2006. *****

- Single molecule imaging: applications in chromatin structure. In Vivo Imaging from Molecule to Organism, Optical Imaging Centre, Erasmus MC, Rotterdam, 23 November 2006. *****

- Single-molecule experiments on chromatin structure and dynamics. MRC Laboratory of Molecular Biology, Cambridge, U.K., 4 april 2007. *****

- Single-molecule experiments on chromatin structure and dynamics. Eurodyna SM Meeting, Institut für Molekularbiologie und Tumorforschung (IMT), Marburg, Duitsland, 12-13 april 2007. *****

- Single-molecule experiments on chromatin structure and dynamics. AMOLF Colloquium, Amsterdam, 16 april 16 2007. *****

- Unraveling chromatin structure and dynamics with single-molecule techniques. MB Biolunch Seminar, Delft, 22 oktober 2007. *****

- Single-molecule Force spectroscopy substantiates a solenoidial structure of 30 nm chromatin fibers MRC Laboratory of Molecular Biology, Cambridge, U.K., 18 Januari 2008. *****

- Posters

Colin Logie:

EMBL 7th transcription meeting: Heidelberg, Aug. 2006 – Poster. *****

Joke van Vugt:

Dutch chromatin meeting IV, Dec. 2005, Kerkrade – Poster. *****

Fourth International Summer School DNA and Chromosomes 2006, Physical and Biological Approaches, Cargèse, FRANCE June 2006 – Poster. *****

ELSO-2007 meeting, Dresden, Septembre 2007 –poster. *****

M. Murawska:

M. Murawska, N. Kunert, E. Kremmer and A.Brehm, Characterisation of Drosophila Chd3 – a putative ATP-dependent chromatin remodeler – presented at the conference “Chomatin-mediated biological decisions”, Marburg 5.-7. October 2006 – Poster.

M. Murawska, Biochemical characterization of ATP-dependent chromatin remodeling by a novel CHD enzyme - dCHD3 – presented at the Workshop “Protein-Nucleic Acid Interactions” of the International Research Training Group Gießen/Marbur-Moscow (GRK1384), Suzdal, June 2007 – poster.

M. Murawska, dCHD3 – a novel ATP-dependent chromatin remodeler – presented at the EMBO Conference on Chromatin and Epigenetics, Heidelberg, May 2007 – poster.

M. Murawska, Characterization of a novel CHD chromatin remodeler – dCHD3 in Drosophila melanogaster- presented at the 2nd Transregio 5 Symposium “Chromatin – Assembly and Inheritance of Functional States” – poster.

Martijn de Jager:

- Mechanism of dMi-2-mediated chromating remodeling. *****
- Annual Dutch Meeting on Molecular and Cellular Biophysics, Lunteren, The Netherlands, 10 and 11 October 2005, poster (de Jager , M., Boer, I. de, Logie, C., Brehm, A., Noort, S.J.Th. van). *****
- Unraveling the mechanism of dMi-2-mediated chromating remodeling. Dag van de Biofysica, Leiden, The Netherlands, 18 November 2005, poster (de Jager , M., Boer Logie, C., Brehm, A., Noort, S.J.Th. van). *****
- Unraveling the mechanism of dMi-2-mediated chromatin remodeling. Single Molecule Meeting, Cambridge, Engeland, 26-29 March 2006. *****
- Unraveling the mechanism of dMi-2-mediated chromatin remodeling. Annual Dutch Meeting on Molecular and Cellular Biophysics, Lunteren, The Netherlands, 9 and 10 October 2006, (de Jager , M., Boer Logie, C., Brehm, A. and Noort, J. van). *****
- AFM studies on the mechanisms of nucleosome repositioning. Annual Dutch Meeting on Molecular and Cellular Biophysics, Veldhoven, 1-2 oktober 2007, poster (de Jager , M., Boer M. Engeholm, J. van Vugt, C. Logie, A. Brehm, T. Owen-Hughes & S.J.T. van Noort). *****

John van Noort:

- AFM studies on the mechanism of nucleosome remodeling by RSC. 52th Annual Meeting of the Biophysical Society, Long Beach. CA, U.S.A., maart 2008, poster. *****
- spFRET spectroscopy on nucleosome dynamics. Annual Dutch Meeting on Molecular and Cellular Biophysics, Lunteren, The Netherlands, 10 and 11 October 2005, poster (Koopmans. W, Logie, C.,

Schmidt. T., Noort, S.J.Th. van). *****

- spFRET spectroscopy on nucleosome dynamics. Dag van de Biofysica, Leiden, The Netherlands, 18 November 2005, poster (Koopmans. W, Boer, I. de, Logie, C., Schmidt, T., Noort, S.J.Th. van). *****

- Single-pair FRET microscopy on nucleosome dynamics. 51th Annual Meeting of the Biophysical Society, Baltimore, MD., U.S.A., maarch 2007, poster (Koopmans. W, C. Logie, T. Schmidt & S.J.T. van Noort). *****

- Forced disassembly of chromatin fibers. Annual Dutch Meeting on Molecular and Cellular Biophysics, Lunteren, The Netherlands, 10 and 11 October 2005, poster (Kruithof, M., Boer, I. de, Logie, C., Noort, S.J.Th. van). *****

- Forced disassembly of chromatin fibers. Dag van de Biofysica, Leiden, The Netherlands, 18 November 2005, poster (Kruithof, M., Boer, I. de, Logie, C., Noort, S.J.Th. van). *****

- Probing nucleosome interaction using dynamic force spectroscopy on single chromatin. 51th Annual Meeting of the Biophysical Society, Baltimore, MD., U.S.A., maart 2007, poster (M. de Jager, S.J.T. van Noort & C. Logie). *****

- Other (*please define*)

Public Outreach:

- Press releases

- National / international Newspaper articles (presenting your CRP or part of your work)

- TV appearance

- Radio appearance

- Other (*please define*)

John van Noort

Fourth International Summer School - DNA and Chromosomes 2006: Physical and Biological Approaches - 1 July 2006 Cargèse, Corsica, FRANCE – Organising committee *****

Colin Logie

Pod-cast at http://www.esf.org/media-centre/podcast-and-video-gallery/podcast-from-seppia.html *****

The Sixth Dutch Chromatin Meeting (Nijmegen -Octobre 2008) – organiser

Alexander Brehm

Conference “Chromatin mediated Biological Decisions”, Marburg, October 2006 – organising committee

Other Activities / Products

- Patents

- Websites

- Other (*please define*)

IMPORTANT: In your list, please identify with an asterisk (*) those publications and contributions in which the EuroDYNA Programme is properly acknowledged.

The control of chromosome structure by cohesion/ condensin complexes

Abstract

All organisms possess sophisticated mechanisms to respond to environmental stress. The robust transcriptional response to osmostress in yeast works through a conserved MAP kinase signaling pathway that leads to a major reprogramming of the cell's transcriptional state. The MAPK Hog1 acts directly at a large number of promoters to induce expression of osmostress-reponse genes, while a less well understood mechanism(s) causes coordinate down-regulation of growth-related genes, such as those encoding ribosomal proteins. Our primary aim will be to characterize and elucidate mechanisms underlying the dynamic changes in chromatin structure and modification states that accompany both the induction and down-regulation of genes following osmostress, using chromatin immunoprecipitation as our standard experimental tool. We will also employ high throughput genetic screens and advanced biochemical methods to understand how the chromatin remodeling / modification machinery interacts with global and gene-specific transcription factors and how these interactions promote dramatic changes in transcription rates. Finally, advanced cell biological methods (e.g. FRAP) will be used to characterize the dynamic nuclear trafficking and chromatin association of specific transcription factors following osmostress. These studies should yield new and important insights into mechanisms by which chromatin modification is linked to highly dynamic changes in transcription and overall nuclear architecture.

Partners

(DFG, FWF, MRC)

Dr Jan-Michael Peters (Project Leader)
IMP, Vienna, Austria

Prof Terence David Allen
Paterson Institute for Cancer Research, Manchester, UK

Dr Roland Eils
German Cancer Research Centre, Heidelberg, Germany

Dr Jan Ellenberg
EMBL, Heidelberg, Germany

Dr Jan Löwe
Medical Research Council, Cambridge, UK

Prof Kim Nasmyth
University of Oxford, UK

A.1. General information about the Collaborative Research Project (CRP)
CRP Number: 03-DYNA–F-29 **CRP Title and Acronym**: **The control of chromosome structure by cohesin/condensin complexes** **Acronym:** cohesin/condensin complexes **Name Project Leader**: Dr. Jan-Michael Peters **Project-related website**: www.univie.ac.at/eurodyna/ **Reporting period**: from 01/03/2005 to 01/03/2008
List all Principal Investigators (PIs), Associated Partners (APs) and Co-operating Partners (CPs) of the CRP
PI 1
name: **Prof. Kim Nasmyth**
PI 2
name: **Dr. Jan Löwe**
PI 3
name: **Dr. Jan Ellenberg**
PI 4
name: **Dr. Roland Eils**
PI 5
name: **Prof. Terence David Allen**
PI 6
name: **Dr. Jan-Michael Peters**

A.3. Report of the CRP in terms of Networking, Training and Dissemination

→ (To be completed by Project Leader with input from CRP members)

6. Networking activities: Of all the networking activities developed during this period, please highlight the most important one for this CRP (in terms of outcome, impact, opportunities for trans-national collaborations and synergy with other European and international initiatives)

In addition to the collaborations described under 2. and the European-added value described under 4. EuroDYNA had close contacts to a network of European scientists working on mitosis through the participation of Eils, Ellenberg, Nasmyth, Peters in the FP6 funded Integrated Project MitoCheck (www.mitocheck.org).

7. Training activities: Please, highlight the most useful training activity (workshop, course or school) undertaken by senior or junior researchers of this CRP

Harder N, Workshop Microscopic Image Analysis with Applications in Biology (MIAAB'2006), Copenhagen, Denmark, 5. Oct. 2006

Harder N, Workshop on Bio-Image Informatics: Biological Imaging, Computer Vision and Data Mining. Santa Barbara/CA, USA, January 17-18, 2008

8. Dissemination activities: What has this CRP done to promote the EuroDYNA Programme more actively and widely? Please, indicate the outcome and impact of the dissemination activities developed during this period

The results obtained with this CRP were disseminated in a number of publications in high-ranking journals and through numerous invited lectures that were given by the principal investigators of this CRP (in particular by Nasmyth, Ellenberg and Peters) at other research institutes or scientific conferences.

A.4 Valuable feedback from this CRP

9. Key contribution of this CRP to the EURODYNA Programme: How has the EuroDYNA Programme benefited from this CRP? Please, provide one example that clearly illustrates your valuable input (whether scientific, networking, training and/or dissemination input)

This CRP added to the visibility of EuroDYNA through participation of internationally well known scientists (for example Nasmyth). This CRP helped to organize the annual meeting in Brno (Peters), and two members of this CRP gave plenary lectures at the annual meetings (Nasmyth, Peters). Work of this CRP on cohesin also contributed to an interest in this topic in other CRPs (see, for example, the exchange between the Peters and Galjart labs described above).

10. Key contribution of the EURODYNA Programme to this CRP: How has your participation in the EuroDYNA Programme influenced your research (in terms of visibility, collaborations, opportunities, ideas)

The EuroDYNA programme facilitated numerous multidisciplinary collaborations between members of this CRP (see 2. and 3. above), stimulated contacts and exchange of ideas between this CRP and members of other CRPs (see 4. above) and made essential contributions to the funding of projects in this CRP through the national funding agencies.

A.5 Follow up related to the CRP and EuroDYNA Programme

11. Follow up: Please provided details of the most important new initiatives (either within a national or an international context) that have been developed as a result of the collaboration of this CRP and the EuroDYNA Programme.

Several collaborations that existed within this CRP will continue in the future: Ellenberg-Peters, Ellenberg-Eils, Peters-Nasmyth and Nasmyth-Löwe. In addition, the Peters and Galjart labs will stay in close contact and plan to collaborate in the future. Kerstin Wendt from the Peters lab is presently in negotiations about a group leader position in the Department in which Niels Galjart works.

A. 5. List of Publications, Dissemination and Outreach Activities undertaken by the CRP

Publications

- Articles

Peer reviewed articles in journals (published, in press or submitted)

Gerlich, D., Hirota, T., Koch, B. Peters, J.-M., and Ellenberg, J. (2006).

Condensin I stabilizes chromosomes mechanically through a dynamic interaction in live cells. *Curr. Biol*. 16, 333-344.

Watrin, E., Schleiffer, A., Tanaka, K., Eisenhaber, F., Nasmyth, K. and Peters, J.-M. (2006). Human Scc4 is required for loading of cohesin onto chromatin, sister chromatid cohesion and progression through mitosis. *Curr. Biol.* **16**, 863-874.

Gerlich, D., Koch, B., Dupeux, F., Peters, J.-M., and Ellenberg, J. (2006). Live cell imaging reveals a stable cohesin-chromatin interaction after but not before DNA replication. *Curr. Biol.* **16**, 1571-1578.

Kueng, S., Hegemann, B., Peters, B.H., Lipp, J.J., Schleiffer, A., Mechtler, K. and Peters, J.-M. (2006). Wapl controls the dynamic association of cohesin with chromatin. *Cell* **127**, 955-967.

Lipp, J.J., Hirota, T., Poser, I. and Peters, J.-M. (2007). Aurora B controls the association of condensin I but not condensin II with mitotic chromosomes. *J. Cell Sci.* **120**, 1245-1255.

Schmitz, J. Watrin, E., Lénárt, P., Mechtler, K., and Peters, J.-M. (2007). Sororin is required for stable binding of cohesin to chromatin and for sister chromatid cohesion in interphase. *Curr. Biol*. **17**, 630-636.

Nakajima, M., Kumada, K., Hatakeyama, K., Noda, T., Peters, J.-M. and Hirota, T. (2007). The complete removal of cohesin from chromosome arms depends on separase. *J. Cell Sci.* **120**, 4188-4196.

Koch, B. Kueng, S., Ruckenbauer, C. and Peters, J.-M. (2007). The Suv39h-HP1 histone methylation pathway is dispensable for enrichment and mitotic protection of cohesin at centromeres in mammalian cells. *Chromosoma*, 117, 199-210

Wendt*; K.S., Yoshida*, K., Itoh*, T., Bando, M., Koch, B., Schirghuber, E., Tsutsumi, S., Nagae, G., Ishihara, K., Mishiro, T., Yahata, K., Imamoto, F., Aburatani, H., Nakao, M., Imamoto, N., Maeshima, K., Shirahige, K. and Peters, J.-M. (2008). Cohesin mediates transcriptional insulation by CTCF. *Nature*, 451,796-801.

Schuh M, Ellenberg J. (2007). Self-organization of MTOCs replaces centrosome function during acentrosomal spindle assembly in live mouse oocytes. *Cell.* 130, 484-498.

Daigle N, Ellenberg J. (2007) LambdaN-GFP: an RNA reporter system for live-cell imaging. *Nat*

Methods. 4(8):633-636.

Mora-Bermúdez F, Gerlich D, Ellenberg J. (2007) Maximal chromosome compaction occurs by axial shortening in anaphase and depends on Aurora kinase. *Nat Cell Biol.* 9(7):822-831.

Neumann B, Held M, Liebel U, Erfle H, Rogers P, Pepperkok R, Ellenberg J. (2006) High-throughput RNAi screening by time-lapse imaging of live human cells. *Nat Methods*. 3(5):385-390.

Ulrich, M., Kappel, C., Beaudouin, J., Hezel, S., Ulrich, J., Eils, R. (2006) Tropical-parameter estimation and simulation of reaction-diffusion models based on spatio-temporal microscopy images. *Bioinformatics* 22, 2709-2710.

Harder N, Eils R, and Rohr K (2008) Automated classification of mitotic phenotypes of human cells using fluorescent proteins, *Methods Cell Biology*, 85, 539-554

Published contributions to international conferences

Harder N, Mora-Bermúdez F, Godinez WJ, Ellenberg J, Eils R, and Rohr K. Determination of Mitotic Delays in 3D Fluorescence Microscopy Images of Human Cells Using an Error-Correcting Finite State Machine. In Proc. IEEE Internat. Symposium on Biomedical Imaging: From Nano to Macro (ISBI'07), Arlington, VA, USA, pages 1044-1047, April 12-15 2007

Kim IH, Godinez W, Harder N, Mora-Bermúdez F, Ellenberg J, Eils R, Rohr K. Compensation of global movement for improved tracking of cells in time-lapse confocal microscopy image sequences. Proc. SPIE Medical Imaging 2007 (MI'07). San Diego, CA/USA. SPIE. Feb. 2007

Kim IH, Yang S, Le Baccon P, Heard E, Kappel C, Chen Y., Spector D, Eils R, Rohr K. Non-rigid temporal registration of 2D and 3D multi-channel microscopy image sequences of human cells. Proc. IEEE International Symposium on Biomedical Imaging: From Nano to Macro (ISBI'07). 2007; 1328-1331

Rohr K, Harder N, and Eils R. Automated Analysis of Spatio-Temporal Cell Microscopy Images: Classification, Tracking, and Registration, Proc. 6th International Congress on Industrial and Applied Mathematics (ICIAM'07), ETH Zürich, University of Zürich, Switzerland, 2007

F. C. Gardiner, T. D. Allen. High Resolution Imaging of Condensing Chromatin by FESEM Structural Cell Biology, Paterson Institute for Cancer Research, Manchester, United Kingdom ASCB San Francisco 2005

F. C. Gardiner, T. D. Allen High Resolution Imaging of Cohesin and Condensin Structures by FESEM ; Structural Cell Biology, Paterson Institute for Cancer Research, Manchester, United Kingdom ASCB San Diego 2006

News & Views-type articles

Watrin, E. and Peters, J.-M. (2006). Cohesin and DNA damage repair. *Exp. Cell Res.* **312**, 2687-2693.

Other articles (*please define*)

Watrin, E. and Peters, J..M. (2007). Molecular biology. How and when the genome sticks together. *Science* **317**, 209-210. (Review)

Mora-Bermúdez F, Ellenberg J. (2007) Measuring structural dynamics of chromosomes in living cells by fluorescence microscopy. *Methods.* 41(2):158-167. (Review)

Presentations in Scientific Meetings

- Oral presentations (indicate invited / keynote talks)

Jan-Michael Peters. "Regulation of sister chromatid cohesion in mammalian cells" Annual Meeting of the American Society of Biochemistry and Molecular Biology (ASBMB)

Washington D.C., USA 28/03 - 02/04 2007

Jan-Michael Peters. "MitoCheck - a global view at mitosis" (Opening plenary lecture) Gordon Research Conference on Cell Growth and Proliferation. Biddeford, MA, USA

23-27/06 2007

Jan-Michael Peters. "Anti-mitotic compounds as tools for basic research and cancer therapy" EMBO Workshop on Drug Action and Chemical Biology in the Post-Genomic Era. Vienna 23-26/08 2007

Jan-Michael Peters. "Functions of cohesin in mammalian cells". EMBO Workshop on Molecular

Mechanisms of Cell Cycle Control in Normal and Malignant Cells" Spetses, Greece

5-8/10 2007

Jan-Michael Peters. "Regulation of sister chromatid cohesion in mammalian cells". Annual Congress of the International Union of Biochemistry and Molecular Biology. Kyoto, Japan. 21-24 May 2006

Harder N, Mora-Bermúdez F, Godinez WJ, Ellenberg J, Eils R, and Rohr K, "Automated analysis of the mitotic phases of human cells in 3D fluorescence microscopy image sequences", Workshop Microscopic Image Analysis with Applications in Biology (MIAAB'2006), Copenhagen, Denmark, 5. Oct. 2006

Rohr K, Gladilin E, Godinez WJ, Harder N, Kim IH, Wörz S, Yang S, and Eils R, "Automated Analysis of Cell Microscopy Images: Registration, Tracking, and Classification", Proc. 6th International ELMI Meeting and Workshop on Advanced Light Microscopy (ELMI'06), Ofir, Portugal, May 30 - June 2, 2006

Harder N, Mora-Bermúdez F, Godinez WJ, Ellenberg J, Eils R, and Rohr K. Automated analysis of cell phenotype screening data using high-throughput cellular imaging. Global Imaging Summit 2006: The Application of Cellular and Molecular Imaging in Drug Discovery. Zurich/Switzerland, December 5-6, 2006

Kim IH, Godinez WJ, Harder N, Mora-Bermúdez F, Ellenberg J, Eils R, Rohr K. Compensation of global movement for improved tracking of cells in time-lapse confocal microscopy image sequences. Proc. SPIE Medical Imaging 2007 (MI'07). San Diego, CA/USA. SPIE. Feb. 2007

Harder N, Mora-Bermúdez F, Godinez WJ, Ellenberg J, Eils R, and Rohr K. Determination of Mitotic Delays in 3D Fluorescence Microscopy Images of Human Cells Using an Error-Correcting Finite State Machine. In Proc. IEEE Internat. Symposium on Biomedical Imaging: From Nano to Macro (ISBI'07), Arlington, VA, USA, pages 1044-1047, April 12-15 2007

Kim IH, Yang S, Le Baccon P, Heard E, Kappel C, Chen Y., Spector D, Eils R, Rohr K. Non-rigid temporal registration of 2D and 3D multi-channel microscopy image sequences of human cells. Proc. IEEE International Symposium on Biomedical Imaging: From Nano to Macro (ISBI'07). 2007; 1328-1331

Rohr K, Harder N, and Eils R. Automated Analysis of Spatio-Temporal Cell Microscopy Images: Classification, Tracking, and Registration, Proc. 6th International Congress on Industrial and Applied Mathematics (ICIAM'07), ETH Zürich, University of Zürich, Switzerland, 2007

Harder N, Mora-Bermúdez F, Godinez WJ, Ellenberg J, Eils R, and Rohr K. Large-scale identification of cellular phenotypes by automated image analysis of live cell arrays. LabAutomation 2007. Palm Springs/CA USA, January 27-31, 2007.

Harder N, Mora-Bermúdez F, Godinez WJ, Ellenberg J, Eils R, and Rohr K. Automatic phenotypic analysis of video microscopy. International Symposium on High Throughput Microscopy for Systems Biology, Heidelberg, Germany, April 28-29, 2007

Harder N, Mora-Bermúdez F, Godinez WJ, Ellenberg J, Eils R, and Rohr K. Automated analysis of mitotic cell nuclei in 3D fluorescence microscopy image sequences. Workshop on Bio-Image Informatics: Biological Imaging, Computer Vision and Data Mining. Santa Barbara/CA, USA, January 17-18, 2008

- Posters

Kappel, C., Ulrich, M., Beaudouin, J., Hezel, S., Ulrich, J., Eils, R. „Quantitative in vivo analysis of protein mobility using the software Tropical“, Conference EuroDYNA, Dynamic nuclear architecture and chromatin function, Mendel Center in Brno, Czech Republic, 12.-14. October 2006

Harder N, Mora-Bermúdez F, Godinez WJ, Ellenberg J, Eils R, and Rohr K, "Automated analysis of the mitotic phases of human cells in 3D fluorescence microscopy image sequences", Proc. 9th Internat. Conf. on Medical Image Computing and Computer-Assisted Intervention (MICCAI'2006), Copenhagen, Denmark, 1.-6. Oct. 2006, Lecture Notes in Computer Science 4190, Part I, R. Larsen, M. Nielsen, and J. Sporring (Eds.), Springer-Verlag Berlin Heidelberg 2006, 840-848

Harder N, Mora-Bermúdez F, Godinez WJ, Ellenberg J, Eils R, and Rohr K. Automated multi-dimensional image analysis for evaluating live cell fluorescence microscopy images. Conference EuroDYNA 2008, Hinxton/UK, 28-31 May 2008

Public Outreach:

- Press releases

- National / international Newspaper articles (presenting your CRP or part of your work)

- TV appearance

- Radio appearance

Other Activities / Products

- Patents

- Websites

- Other (*please define*)

IMPORTANT: In your list, please identify with an asterisk (*) those publications and contributions in which the EuroDYNA Programme is properly acknowledged.

Environmental stress-induced dynamic modulation of chromatin structure, gene expression and nuclear architecture in yeast

Abstract

All organisms possess sophisticated mechanisms to respond to environmental stress. The robust transcriptional response to osmostress in yeast works through a conserved MAP kinase signaling pathway that leads to a major reprogramming of the cell's transcriptional state. The MAPK Hog1 acts directly at a large number of promoters to induce expression of osmostress-reponse genes, while a less well understood mechanism(s) causes coordinate down-regulation of growth-related genes, such as those encoding ribosomal proteins. Our primary aim will be to characterize and elucidate mechanisms underlying the dynamic changes in chromatin structure and modification states that accompany both the induction and down-regulation of genes following osmostress, using chromatin immunoprecipitation as our standard experimental tool. We will also employ high throughput genetic screens and advanced biochemical methods to understand how the chromatin remodeling / modification machinery interacts with global and gene-specific transcription factors and how these interactions promote dramatic changes in transcription rates. Finally, advanced cell biological methods (e.g. FRAP) will be used to characterize the dynamic nuclear trafficking and chromatin association of specific transcription factors following osmostress. These studies should yield new and important insights into mechanisms by which chromatin modification is linked to highly dynamic changes in transcription and overall nuclear architecture.

Partners

(SNF, FWF)

Dr David Shore (Project Leader)
University of Geneva, Switzerland

Dr Gustav Ammerer
University of Vienna, Austria

Dr Matthias Peter
Swiss Federal Institute of Technology (ETH), Zurich, Switzerland

Associated Partner:

Dr Francesc Posas
University of Pompeu Fabra, Barcelona, Spain

Part A. Collaborative Research Project (CRP)

→ (To be completed by Project Leader)

A.1. General information about the Collaborative Research Project (CRP)

CRP Number: 03-DYNA–F-32

CRP Title and Acronym: **Environmental stress-induced dynamic modulation of chromatin structure, gene expression and nuclear architecture in yeast / Chromatin dynamics of transcriptional stress response in yeast**

Name Project Leader: Prof. David Shore

Project-related website: http://www.molbio.unige.ch/shore/index.php

Reporting period: from 01/04/2005 to 01/04/2008 (Please report for the whole duration of your project)

List all Principal Investigators (PIs), Associated Partners (APs) and Co-operating Partners (CPs) of the CRP

PI 1
name: **David Shore**
PI 2
name: **Gustav Ammerer**
PI 3
name: **Matthias Peter**
AP 1
name: **Francesc Posas**

A.3. Report of the CRP in terms of Networking, Training and Dissemination

→ (To be completed by Project Leader with input from CRP members)

Please provide a concise and clear description of the networking, training and dissemination activities undertaken by this CRP, addressing the following issues:

6. Networking activities: Of all the networking activities developed during this period, please highlight the most important one for this CRP (in terms of outcome, impact, opportunities for trans-national collaborations and synergy with other European and international initiatives)

Probably the most important networking activities were the informal meetings held in Vienna together with the CRP headed by Pavel Kovarik (Vienna) on "Control of stress and interferon regulated gene expression", as well as the formal EuroDYNA meeting in Brno. These relatively informal visits with the involved laboratories ensured an active exchange during the entire funding period. For example, visits by two members of the Shore lab (V. Martin-Sanchez and H. Lempiainen) led to direct collaborations with the Ammerer lab on phosphopeptide analysis of ribosomal protein gene activators (Ifh1 and Sfp1). The EuroDYNA meeting organized in Brno led directly to a collaboration between the Shore lab, Ammerer's group and a colleague colleague of his in Vienna, Christoph Schüller, on the role of TORC1 and Sfp1 in the cellular response to arsenic. In addition, Dr. Eulàlia de Nadal from the Posas laboratory worked for several month in Zürich, and used the automated screening setup to isolate mutants defective for various aspects of stress signaling and chromatin regulation. The quantitative single cell analysis project also profited from a close interaction with the EU-network QUASI. Finally, because of the unexpected link of Urm1p to RNA-modification, Peter and colleagues initiated collaborations with the ESF RNAQuality project.

7. Training activities: Please, highlight the most useful training activity (workshop, course or school) undertaken by senior or junior researchers of this CRP

EuroDYNA Workshop, Brno, September, 2006. QUASI meetings: Göteborg June, 2005, Lenzerheide, January, 2006, Göteborg, June, 2006, Rostock, September, 2006, Vienna, January, 2007, Barcelona May 2007. ESF Yeast Transcription Meeting, Barcelona, June 2006.

8. Dissemination activities: What has this CRP done to promote the EuroDYNA Programme more actively and widely? Please, indicate the outcome and impact of the dissemination activities developed during this period

Attendance and talk at the ESF RNAQuality meeting organized in Denmark by Torben Jensen in September, 2007. This led to several productive interactions that may soon lead to direct collaborations.

Each member of the CRP travels regularly to present invited talks (either in meetings or University seminar programs) and this has also been important to the dissemination of the CRP's activities throughout Europe and abroad.

A.4 Valuable feedback from this CRP

→ (To be completed by Project Leader with input from CRP members)

Please, provide a <u>concise and clear answer</u> to these questions:

9. Key contribution of this CRP to the EURODYNA Programme: How has the EuroDYNA Programme benefited from this CRP? Please, provide one example that clearly illustrates your valuable input (whether scientific, networking, training and/or dissemination input)

Scientific: demonstration in several specific examples of the relevance of chromatin modifications to regulation of gene expression (e.g. Hog1 recruitment of RSC to promote transcriptional elongation and role of INO80 in restoring nucleosome structure at stress-induced genes). Networking: productive interactions with both the "Control of stress and interferon regulated gene expression" CRP headed by P. Kovarik as well as Colin Logie in the "Chromatin higher order dynamics" CRP, which has resulted in a collaborative manuscript currently under review.

10. Key contribution of the EURODYNA Programme to this CRP: How has your participation in the EuroDYNA Programme influenced your research (in terms of visibility, collaborations, opportunities, ideas)

I think that for all of the groups the specific collaborations referred to above have clearly allowed each groups' work to progress more rapidly and in directions that would not have been possible in the absence of the EuroDYNA-sponsored collaborative scheme. The Shore lab has particularly benefited from the interaction with the other groups, all of who have worked closely together in the past, by gaining a rapid and intensive introduction to the field, which he (Shore) entered relatively recently. Apart from funding the work itself, EuroDYNA support for lab visits by postdocs and students, and travel support for joint informal network meetings has been particularly useful.

A.5 Follow up related to the CRP and EuroDYNA Programme

→ (To be completed by Project Leader with input from CRP members)

11. Follow up: Please provided details of the most important new initiatives (either within a national or an international context) that have been developed as a result of the collaboration of this CRP and the EuroDYNA Programme.

Three of the four groups will continue their direct collaboration through UNICELLSYS, a EU (FP7) project. The Shore lab will soon begin a collaborative project in the context of the Swiss "SystemsX" programme, designed to support work in the area of "Systems Biology". This project will develop from work initiated in the EuroDYNA CRP.

A. 5. List of Publications, Dissemination and Outreach Activities undertaken by the CRP

→ (To be completed by Project Leader with input from CRP members)

Publications

- Articles

Peer reviewed articles in journals (published, in press or submitted)

*Zapater M, Sohrmann M, Peter M, Posas F, de Nadal, E. (2007) "Selective requirement for SAGA in Hog1-mediated gene expression depending on the severity of the external osmostress conditions". *Mol Cell Biol*. **27**:3900-10.

Kraft, C., Deplazes, A., Sohrmann, M, and Peter, M. (2008) "Mature ribosomes are selectively degraded on

starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease". *Nature Cell Biology*, in press.

*Leidel, S., Pedrioli, P., Brost, R., Bucher, T., Costanzo, M., Aebersold, R., Charles Boone, C., Kay Hofmann, K. and Matthias Peter, M. (2008) "The ubiquitin-related modifier Urm1p functions as a sulfur-carrier in thiolation of eukaryotic tRNA". *Nature*, in revision.

*Glòria Mas, Eulàlia de Nadal, Reinhand Dechant, María Luisa Rodríguez de la Concepción, Colin Logie, Silvia Jimeno-González, Sebastián Chávez, Gustav Ammerer and Francesc Posas. (2008) "Hog1 MAPK targets the RSC complex to mediate eviction of H3-K4 modified nucleosomes in osmoresponsive genes". Submitted.

*Lempiäinen, H., Aino U., Urban, J., Dohnal, I., Ammerer, G., Loewith, R.L. and Shore, D. (2008) "Direct Interaction of TORC1 with the Ribosome Biogenesis Gene Activator Sfp1 Reveals Novel Features of TOR signaling". Submitted.

*Hosiner, D., Lempiäinen, H., Reiter, W., Urban, J., Loewith, R.L., Ammerer, G., Rudolf Schweyen, R., Shore, D. and Schüller, C. (2008) "Arsenic inhibits the Saccharomyces cerevisiae TORC1 kinase". Submitted.

*Paskova L, E Klopf, G Mas, A Petryshyn, F Posas, U Wintersberger, G Ammerer and C Schüller (2008). "INO80 restores nucleosome architecture after stress induced eviction in yeast". Submitted.

Published contributions to international conferences

News & Views-type articles

Other articles (*please define*)

- Books

As editor(s)

F. Posas (2008) "Stress-Activated Protein Kinases" F. Posas and A.R. Nebreda (Eds.) *Topics in Current Genetics*, Vol. 20, Springer

As author(s)

- Other (*please define*; *e.g.*, data products, videos)

Presentations in Scientific Meetings

- Oral presentations (indicate invited / keynote talks)

Gordon Conference "Ubiquitin and Ubiquitin-like proteins", invited speaker (M. Peter)

Cold Spring Harbor Meeting, "Ubiquitin", invited speaker (M. Peter)

OIST Workshop on Yeast Signaling (March 2008), invited speaker together with Dr. Serge Pelet (M. Peter)

Joining-Forces Workshop, ETH Zürich, invited speaker (M. Peter)

Workshop on Autophagy, Lenzerheide, Switzerland, organizer (M. Peter)

ESF Meeting on “Yeast Transcription” (June, 2006). Invited speakers (F. Posas and D. Shore)

Numerous invited oral presentations at Universities and Research Institutes (all PIs)

- Posters

- Other (*please define*)

Public Outreach:

- Press releases

- National / international Newspaper articles (presenting your CRP or part of your work)

- TV appearance

- Radio appearance

- Other (*please define*)

Other Activities / Products

- Patents

- Websites

IMPORTANT: In your list, please identify with an asterisk (*) those publications and contributions in which the EuroDYNA Programme is properly acknowledged.