9th TRR81 PhD Minisymposium

„Kinases as regulators of chromatin structure and transcription"

Friday, 20th of November 2015, 13:00h

Institute of Molecular Biology and Tumor Research (IMT)

Philipps-University of Marburg, Big Lecture Hall

Confirmed speakers:

Sung-Bau Lee

Biotech Research & Innovation Centre (BRIC), University of Copenhagen (Anja Groth lab)

Nikolaus Watson

Institute for Cell and Molecular Biosciences, University of Newcastle (Jonathan Higgins lab)

Guillermo Pablo Vicent

Centre for Genomic Regulation (CRG), Barcelona (Miguel Beato del Rosal lab)

Carme Solé

Department of Experimental and Health Sciences, University of Barcelona (Francesc Posas Garriga lab)

Alexandra Stützer

Department of Chromatin Biochemistry, Max Planck Institute for Biophysical Chemistry, Göttingen (Wolfgang Fischle lab)

Local organizers: Jana Haas & Markus Seibert (Lienhard Schmitz lab, JLU Gießen)
Sponsoring: DFG – TRR81 (Chromatin Changes in Differentiation and Malignancies)

Program:

13:00h – 13:15h: *Welcome and opening remarks*

Markus Seibert and Jana Haas (Justus Liebig University of Giessen, Germany)

13:15h – 14:00h: **Sung-Bau Lee**

“Tousled-like kinase 2 governs chromatin assembly and replication fork integrity”

14:00h – 14:45h: **Nikolaus Watson**

“An unbiased high-throughput screening approach for identifying novel histone kinases in mitosis”

14:45h – 15:15h: *Snacks and refreshments*

15:15h – 16:00h: **Guillermo Pablo Vicent**

“Role of chromatin dynamics on hormone-dependent gene regulation in breast cancer cells”

16:00h – 16:45h: **Carme Solé**

“Control of gene expression by stress-activated protein kinases (SAPKs)”

16:45h – 17:15h: *Coffee break*

17:15h – 18:00h: **Alexandra Stützer**

“Modulation of DNA contacts by linker histones and post-translational modifications determines the mobility and modifiability of H3 tails in nucleosomes”

19.00h: *Speakers dinners*

Sung-Bau Lee

Biotech Research and Innovation Centre (BRIC), University of Copenhagen

Tousled-like kinase 2 governs chromatin assembly and replication fork integrity

DNA sequence and epigenetic information embedded in chromatin must be faithfully transmitted to daughter cells during cell division. This is fundamental for the development of complex multi-cellular organisms and disease avoidance throughout life. Whereas signaling networks that control genome integrity have been extensively characterized, the mechanisms that ensure duplication of chromatin are still poorly understood. In this study, we identify Tousled-like kinase 2 (TLK2) as a factor required for chromatin assembly and replication fork maintenance. TLKs are a family of kinases highly active in S phase and directly regulated by DNA integrity checkpoints. Loss of TLK function in plants, flies and worms leads to widespread chromosomal defects, developmental abnormalities and lethality. However, the functions of TLKs in mammals remain relatively unexplored. The primary established TLK target is the histone chaperone ASF1, key player in chromatin assembly at the replication fork. We recently reported that TLK phosphorylation of ASF1 enhances its binding to histone H3-H4 and downstream chaperones and thus promotes histone provision. Here we show that TLK2 activity is required for DNA replication and fork integrity. TLK2 depletion leads to single-stranded DNA accumulation, impaired nucleosome assembly and fork stalling concomitant with increased origin firing. Moreover, sustained TLK2 deficiency gives rise to replication-dependent DNA damage and p53-dependent cell cycle arrest in G1. We further show that DNA double-stranded breaks are rapidly generated when both TLK2 and checkpoint activities are compromised. Our results reveal a critical role of TLK2 in chromatin replication and identify a synergistic lethal relationship between replication-coupled chromatin assembly and checkpoint signaling.

Nikolaus Watson

Institute for Cell and Molecular Biosciences, Newcastle University

An unbiased high-throughput screening approach for identifying novel histone kinases in mitosis

Mitotic histones are extensively phosphorylated on a number of residues and in specific chromosomal locations by mitotic kinases. Several of these modifications have key roles in the molecular mechanisms used by the cell to ensure accurate chromosome segregation. Notable among these are the centromeric modification on H3T3 which has been shown to have an integral role in regulating the attachment of spindle microtubules, and H3S10 phosphorylation which appears to regulate Heterochromatin Protein-1 association with chromosomes.

Another centromere specific modification on H3T11 has been described but investigations into its role in mitosis have been impeded by our inability to perform loss of function analysis because the kinase responsible for it remains unknown.

In order to address this problem we have developed a high-throughput RNAi screening approach utilising high-content microscopy which allows us to rapidly screen genes from the human kinome for effects on the abundance of a given histone modification during mitosis. The implementation and early results generated from this approach will be presented.

Guillermo Pablo Vicent

Centre for Genomic Regulation (CRG), Barcelona

Role of chromatin dynamics on hormone-dependent gene regulation in breast cancer cells

Eukaryotic cells interpret environmental information *via* receptors and signalling networks that coincide in the cell nucleus to adjust an integrate the response in form of a defined pattern of gene expression.

A close chromatin conformation precludes gene expression and genes activated by external cues have to overcome a repressive basal state by locally changing chromatin structure to a more open state. Although much is known about hormonal gene activation, how basal repression of regulated genes is targeted is not well understood. On the other hand, steroid hormones regulate gene expression by interaction of their receptors with hormone-responsive elements on DNA or with other transcription factors, but they can also activate cytoplasmic signaling cascades. How these signaling pathways impact on chromatin is not well understood.

Using as an example the response to progestins (Pg) of T47D breast cancer cells we found that rapid Erk activation by progestins leads to phosphorylation of the progesterone receptor (PR), activation of the kinase MSK1 and recruitment of a complex of the three proteins to progesterone-target promoters[1]. In addition, we found in breast cancer cells, that the unliganded progesterone receptor (uPR) binds genomic sites and targets a repressive complex containing HP1γ (heterochromatin protein 1γ), LSD1 (lysine-specific demethylase 1), HDAC1/2, CoREST (corepressor for REST [RE1 {neuronal repressor element 1} silencing transcription factor]), KDM5B, and the RNA SRA (steroid receptor RNA activator) to 20% of hormone-inducible genes, keeping these genes silenced prior to hormone treatment. Upon hormonal treatment, the HP1γ –LSD1 complex is displaced from these constitutively poorly expressed genes as a result of rapid phosphorylation of histone H3 at Ser 10 mediated by the kinase MSK1, which is recruited to the target sites by the activated PR[2-3]. Displacement of the repressive complex enables the loading of coactivators needed for chromatin remodeling and activation of this set of genes, including genes involved in apoptosis and cell proliferation. These results highlight the importance of the unliganded PR in hormonal regulation of breast cancer cells.

Inhibition of MSK1 activity or its depletion by MSK1 short hairpin RNAs (shRNAs) specifically abrogates cell proliferation in response to E2 or progestins. MSK1 activity is required for the transition from the G1- to the S-phase of the cell cycle and inhibition of MSK1 compromises both estradiol- and progestin-dependent induction of cell cycle genes. ChIP-seq experiments identified binding of MSK1 to progesterone receptor-binding sites associated with hormone-responsive genes. MSK1 recruitment

to epigenetically defined enhancer regions supports the need of MSK1 as a chromatin remodeler in hormone-dependent regulation of gene transcription. In agreement with this interpretation, expression of a histone H3 mutated at S10 eliminates the hormonal effect on cell proliferation and on induction of relevant target genes. E2- or progestin-dependent growth of T47D cells xenografted in immunodefficient mice is inhibited by depletion of MSK1, indicating that our findings are not restricted to cultured cells, and that MSK1 plays an important role for hormone-dependent breast cancer growth in a more physiological context.

References:

[1] Vicent *et al.,* (2006), *Mol Cell 24*, 367-81.

[2] Vicent *et al.,* (2011), *Genes Dev 25*, 845-62.

[3] Vicent *et al.,* (2013), *Genes Dev 27*, 1179-97

Carme Solé

Department of Experimental and Health Sciences, University of Barcelona

Control of gene expression by stress-activated protein kinases (SAPKs)

Exposure of yeast cells to high osmolarity results in the activation of the p38-related Hog1 Stress-Activated Protein Kinase (SAPK), which is required to generate a set of osmoadaptive responses. Adaptation to stress requires the induction of a large number of genes that is highly dependent on the presence of Hog1. The SAPK controls several steps of the transcription process upon stress. At initiation, Hog1 not only directly phosphorylates several transcription factors to alter their activities, but also associates at stress-responsive promoters through such transcription factors. Once at promoters, Hog1 serves as a platform to recruit general transcription factors, chromatin modifying activities and RNA Pol II. In addition, Hog1 plays a role in elongation. Genome wide analyses have shown that upon stress there is a global redistribution of RNA Pol II associated to Hog1 targeted loci. The presence of Hog1 at chromatin is critical for chromatin reorganization, which facilitates strong gene induction upon stress. In addition, Hog1 associates and controls the induction of a novel set of lncRNAs in response to osmostress. One of the genes expressing a stress-induced lncRNA in antisense orientation is CDC28, the CDK1 kinase that controls the cell cycle in yeast. Induction of the CDC28 lncRNA permits the increase on the levels of Cdc28 allowing cells to re-entry more efficiently cell cycle after stress. Elucidating the control of gene expression by the Hog1 SAPK should help to understand how eukaryotic cells implement a massive and rapid change on their transcriptional capacity in response to adverse conditions.

Reference:

Carme Solé, Mariona Nadal, Eulalia de Nadal & Francesc Posas, Control of gene expression by stress-activated protein kinases (SAPKs)

Alexandra Stützer

Department of Chromatin Biochemistry, Max Planck Institute for Biophysical Chemistry, Göttingen

Modulation of DNA contacts by linker histones and post-translational modifications determines the mobility and modifiability of H3 tails in nucleosomes

Post-translational histone modifications and linker histone incorporation regulate chromatin structure and genome activity. How these systems interface on a molecular level is unclear. Using biochemistry and NMR spectroscopy we deduced mechanistic insights into the modification behavior of N-terminal histone H3 tails in different nucleosomal contexts. We find that linker histones generally inhibit modification of different H3 sites and reduce H3 tail dynamics in nucleosomes. The effects are caused by modulations of electrostatic interactions of the H3 tail with linker DNA and largely depend on the C-terminal domain of linker histones. In agreement, linker histone occupancy and H3 tail modifications segregate on a genome-wide level. Charge-modulating modifications, such as phosphorylation and acetylation weaken the transient H3 tail-linker DNA interactions, increase H3 tail dynamics and, concomitantly, enhance general modifiability. We propose that alterations of H3 tail-linker DNA interactions by linker histones and charge-modulating modifications execute basal control mechanisms of chromatin function.