# 8 th TRR81 PhD Minisymposium

# " HDACs, HATs & gene regulation"

## Wednesday, 3rd of June, 2015
## 11:00 am

**Venue:**

**Philipps-Universität Marburg**
**Institute of Molecular Biology and Tumor Research (IMT)**
**(Big Lecture Hall)**

## Invited speakers:

**Christopher Millard,**
Department of Biochemistry, University of Leicester *(John Schwabe lab)*
**Jarrett Renn Remsberg,**
Perelman School of Medicine, University of Pennsylvania, Philadelphia *(Mitchell Lazar lab)*
**Tiago Fidalgo Batista,**
IGBMC, Strasburg *(Laszlo Tora lab)*
**Renato Ostuni,**
IFOM-IEO- European Institute of Oncology, Milan *(Gioacchino Natoli lab)*
**Mirjam Moser,**
MFPL – Max F. Perutz Laboratories, Department of Medical Biochemistry, Vienna *(Christian Seiser lab)*

**Local organizer: Francesca Ferrante (Tilman Borggrefe lab)**
**Sponsoring: DFG – TRR81 (Chromatin Changes in Differentiation and Malignancies)**

# Program:

11:00h – 11:15h: ***Welcome, Introduction***
Francesca Ferrante, Chair (JLU, University of Giessen, Germany)

11:15h – 12:00h: **Christopher Millard**
"Insight into the structure and function of Class I HDAC corepressor complexes"

12:00h – 12:45h: **Mirjam Moser**
"HDAC1 and HDAC2 – twins or cousins?"

12:45h – 14:15h: ***Lunch break***

14:15h – 15:00h: **Jarrett Renn Remsberg**
"Characterizing the Mechanism of Histone Deacetylase 3 Function *In Vivo* "

15:00h – 15:45h: **Tiago Fidalgo Batista,**
"The role of the SAGA complex in transcription regulation"

15:45h – 16:15h: ***Coffee Break***

16:15h – 17:00h: **Renato Ostuni**
"Genomic principles of macrophage activation and plasticity"

19.00h: **Speakers dinner**

**Local organizer: Francesca Ferrante (Tilman Borggrefe lab)**
**Sponsoring: DFG – TRR81 (Chromatin Changes in Differentiation and Malignancies)**

**Christopher Millard**
Department of Biochemistry, University of Leicester *(John Schwabe lab)*

## Insight into the structure and function of Class I HDAC corepressor complexes

Histone deacetylases (HDACs) are a family of enzymes that mediate changes in the acetylation state of histones and numerous other non-histone proteins. Class I HDACs are assembled into large protein complexes that play a key role in regulating gene transcription. HDACs 1, 2 and 3 are recruited to distinct complexes: HDAC3 is exclusively recruited to the SMRT/NCoR complex, whereas, HDAC1 and HDAC2 are recruited to the NuRD, Sin3A, MiDAC and CoREST complexes.

We are interested in understanding the specific assembly and mechanism of action of class I HDAC complexes. We express these complexes in mammalian cells and this approach has allowed us to produce milligram quantities of natively assembled complexes for structural and functional studies. We are currently using crystallography, SAXS and chemical crosslinking to determine the molecular architecture of these complexes.

As part of these studies we have recently solved the structure of HDAC1 bound to Metastasis Associated Protein 1 (MTA1) from the NuRD complex. The ELM2-SANT domain of MTA1 is an HDAC recruitment domain that dimerizes to bring two HDACs into close proximity. Functional studies show that inositol phosphates are key regulators of HDAC activity and the mechanism of regulation is common to all class I HDAC complexes.

*Millard CJ, Watson PJ, Celardo I, Gordiyenko Y, Cowley SM, Robinson CV, Fairall L & Schwabe JWR. 2013 Class I HDACs Share a Common Mechanism of Regulation by Inositol Phosphates. Molecular Cell* ***51*** *1–11.*

*Watson PJ, Fairall L, Santos GM & Schwabe JWR. 2012 Structure of HDAC3 Bound to Co-repressor and Inositol Tetraphosphate. Nature* ***481*** *335–340.*

**Local organizer: Francesca Ferrante (Tilman Borggrefe lab)**
**Sponsoring: DFG – TRR81 (Chromatin Changes in Differentiation and Malignancies)**

**Mirjam Moser**
MFPL – Max F. Perutz Laboratories, Department of Medical Biochemistry, Vienna *(Christian Seiser lab)*

## HDAC1 and HDAC2 – twins or cousins?

During development, reversible epigenetic mechanisms including histone modifications modulate gene expression in a tightly controlled manner to ensure faithful differentiation and correct cell fate decisions. The class I histone deacetylases HDAC1 and HDAC2 are highly homologous and are usually found as catalytic components of multimeric co-repressor complexes. They are regarded as crucial regulators of proliferation and differentiation. Originating from a gene duplication, they have overlapping, but also isoform-specific functions in different cell types. Loss-of-function studies have shown that HDAC1 is required for mouse embryonic development, while HDAC2-deficient mice are partially viable. In contrast, conditional ablation of either HDAC1 or HDAC2 in different cell types results in no strong phenotype.

During the last years our lab has focused on the impact of HDAC1 and HDAC2 during the development of epidermis (1), brain (2) and T cells (3). The redundant functions of HDAC1 and HDAC2 are visible upon simultaneous deletion of both enzymes, which leads to DNA damage and apoptosis and is incompatible with normal cellular proliferation and development. Since the individual contributions of HDAC1 and HDAC2 are partially masked by the up-regulated paralog in single knock-out mice, we expressed single alleles of either *Hdac1* or *Hdac2* in the absence of the respective paralog to reveal isoform-specific functions. Importantly, deletion of three of the four *Hdac1/Hdac2* alleles has different consequences depending on the remaining allele and the affected organ. A single *Hdac2* allele is sufficient for normal brain development, whereas mice with a single *Hdac1* allele show premature progenitor differentiation and die shortly after birth. On the other hand, mice with a single allele of *Hdac1* in the epidermis display normal epidermal development and homeostasis, whereas expression of a single *Hdac2* allele in the absence of HDAC1 leads to severe developmental defects including hair loss, hyperkeratosis and increased proliferation. Our studies demonstrate that HDAC1 and HDAC2 are indispensable for the maintenance of chromatin integrity and reveal distinct isoform-specific functions for HDAC1 and HDAC2 in brain and epidermis.

**Local organizer: Francesca Ferrante (Tilman Borggrefe lab)**
**Sponsoring: DFG – TRR81 (Chromatin Changes in Differentiation and Malignancies)**

*Winter, Moser et al. (2013) Divergent roles of HDAC1 and HDAC2 in the regulation of epidermal development and tumorigenesis. EMBO J. 2013 Dec 11;32(24):3176-91.*

*Hagelkruys et al. (2014) A single allele of Hdac2 but not Hdac1 is sufficient for normal mouse brain development in the absence of its paralog. Development. 2014 Feb;141(3):604-16.*

*Boucheron et al. (2014) CD4(+) T cell lineage integrity is controlled by the histone deacetylases HDAC1 and HDAC2. Nat Immunol. 2014 May;15(5):439-48.*

**Local organizer: Francesca Ferrante (Tilman Borggrefe lab)**
**Sponsoring: DFG – TRR81 (Chromatin Changes in Differentiation and Malignancies)**

**Jarrett Renn Remsberg**
Perelman School of Medicine, University of Pennsylvania, Philadelphia *(Mitchell Lazar lab)*

## Characterizing the Mechanism of Histone Deacetylase 3 Function *In Vivo*

Transcriptional responses governed by nuclear receptors are mediated by an array of corepressor and coactivator complexes. Histone deacetylase 3 (HDAC3) is a core component of the NCoR/SMRT transcriptional repressor complex, whose deletion causes profound hepatic steatosis in mouse liver. To better understand the mechanistic functions of HDAC3, we interrogated nuclear and chromatin linked HDAC3-associated proteins in mouse liver using cross linking followed by immunoprecipitation mass spectrometry. This technique, coupled with conditional knockout models and next generation sequencing, provides a powerful system to elucidate networks of nuclear and chromatin associated proteins. Transcription factors, basal transcriptional machinery, and coregulators were found to coprecipitate with HDAC3 in mouse liver nuclei, and a subset of these associations was lost when NCoR was deleted. Understanding the complete set of HDAC3-interacting proteins, and those that are NCoR-dependent or independent, should shed light on the mechanisms by which HDAC3 normally maintains hepatic metabolic homeostasis and prevents fatty liver.

*Jarrett R. Remsberg*, Sean M. Armour*, Simone Sidoli, Zheng Sun, Benjamin A. Garcia, and Mitchell A. Lazar*

**Local organizer: Francesca Ferrante (Tilman Borggrefe lab)**
**Sponsoring: DFG – TRR81 (Chromatin Changes in Differentiation and Malignancies)**

**Tiago Fidalgo Batista**
IGBMC, Strasburg *(Laszlo Tora lab)*

## The role of the SAGA complex in transcription regulation

In the cell nucleus, DNA is wrapped around histone proteins to form nucleosomes, the basic unit of chromatin, which limit the access of transcription machinery to the DNA molecule. Indeed, post-translational modifications of histones regulate chromatin states and thus influence proper transcriptional regulation, cell differentiation and development.

The SAGA (Spt-Ada-Gcn5 acetyltransferase) co-activator complex contains distinct chromatin-modifying activities and is recruited by DNA-bound activators to regulate the expression of a small subset of genes. Surprisingly, recent studies revealed little overlap between genome-wide SAGA-binding profiles and changes in gene expression upon depletion of subunits of the complex.

As indicators of SAGA recruitment on chromatin, we monitored the genome-wide distribution of histone H3K9 acetylation and H2B ubiquitination, which are respectively deposited or removed by SAGA. Changes in these modifications after inactivation of the corresponding enzyme disclosed that SAGA acetylates the promoters and deubiquitinates the transcribed region of all expressed genes, both in yeast and human cells. In agreement with this broad distribution, we show that SAGA plays a critical role for RNA polymerase II recruitment at all expressed genes.

Moreover, and to understand the transcriptional impact of SAGA depletion, we demonstrated that loss of the co-activator induced a strong decrease of mRNA synthesis at all tested genes, through quantification of newly synthesized RNA. Finally, comparative dynamic transcriptome analysis revealed that all genes showed impaired transcription upon SAGA subunits' deletion, which is compensated by an overall decrease on the decay rate. Finally, our most recent work discloses a new function for SAGA as a bone fide cofactor for all RNA polymerase II transcription.

**Local organizer: Francesca Ferrante (Tilman Borggrefe lab)**
**Sponsoring: DFG – TRR81 (Chromatin Changes in Differentiation and Malignancies)**

**Renato Ostuni**
IFOM-IEO- European Institute of Oncology, Milan *(Gioacchino Natoli lab)*

## Genomic principles of macrophage activation and plasticity

Macrophages play crucial roles in many immune and non-immune processes. Recently, the genomic principles underlying their differentiation, plasticity and activation have begun to be highlighted. By applying next generation sequencing (NGS)-based genomic technologies such as ChIP-Seq, RNA-Seq, we have identified regulatory principles controlling macrophage activation in response to environmental signals. Specifically, the molecular mechanisms underlying the genomic cross-talk between multiple and opposing environmental signals will be presented. Genomic approaches can thus lead to unbiased identification of mechanisms underlying key biological processes.

**Local organizer: Francesca Ferrante (Tilman Borggrefe lab)**
**Sponsoring: DFG – TRR81 (Chromatin Changes in Differentiation and Malignancies)**