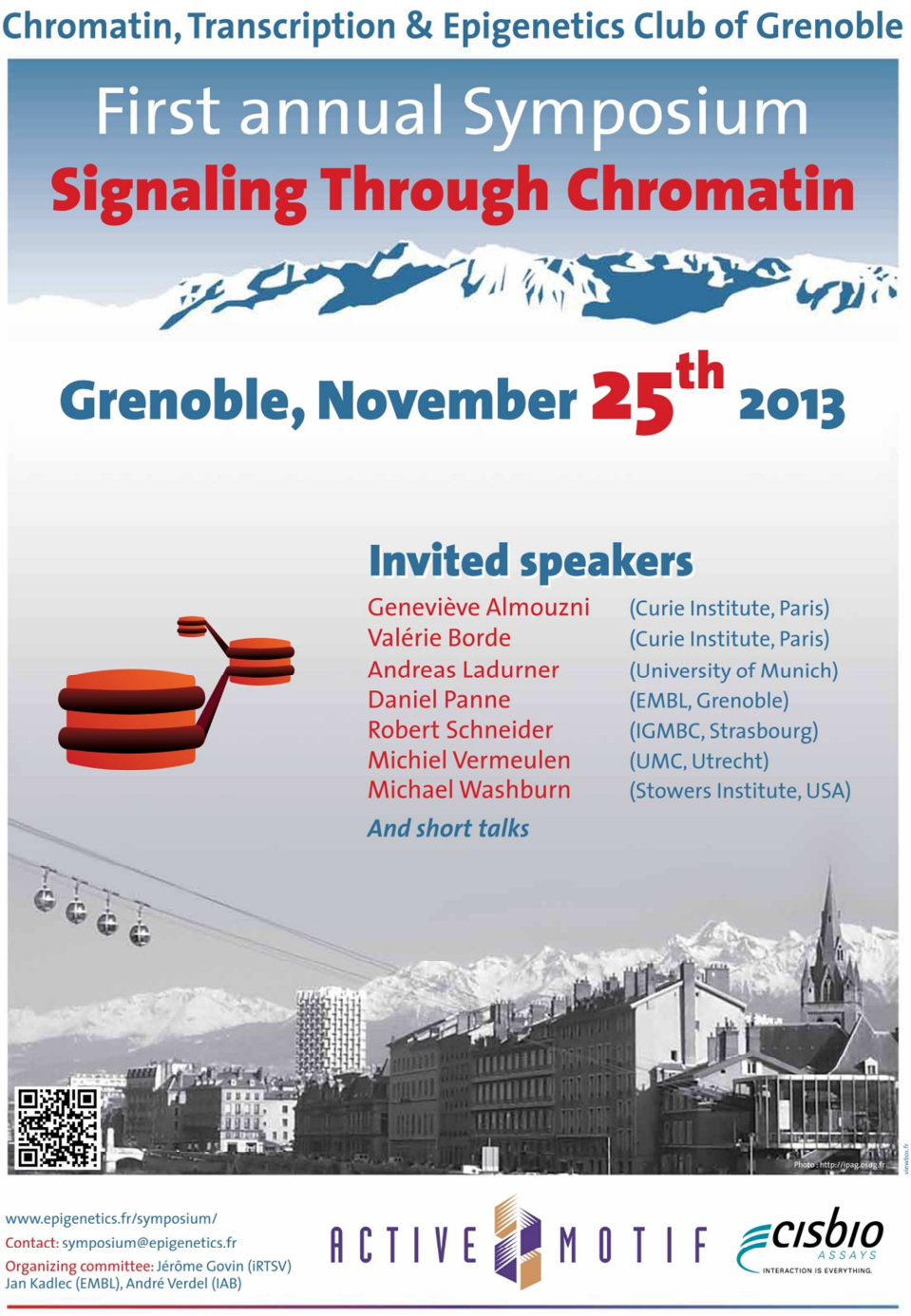

The **Grenoble scientific community** has been demonstrating a growing interest in the field of **chromatin dynamics and epigenetics**. The success and feedbacks have been incredibly good. This is naturally leading us in organizing this symposium in the Fall 2013, on **November 25th 2013**. It will be located at the **Maison Minatec.**

This symposium will focus on the theme "Signaling Through Chromatin". Over the last 10-15 years both exploratory and applied research studies demonstrated that chromatin is a substrate at the crossroads of the different major processes and events of a cell life, including gene expression and the associated regulation mechanisms, as well as cell fate and the balance between proliferation / differentiation. The symposium will cover innovative aspects of epigenetics signaling.

The symposium will be composed of three sessions, covering the area of chromatin dynamics and its link to several DNA-based processes such as transcription, DNA replication, repair and recombination. Two sessions are orientated towards the use of interdisciplinary approaches, such as quantitative proteomics and X-Ray crystallography, to study fundamental aspects of chromatin-based signaling.

The organization committee J.Govin (iRTSV), J.Kadlec (EMBL) and A.Verdel (IAB)

First annual Symposium

Chromatin

Transcription and Epigenetics in Grenoble

Signaling Through Chromatin

Grenoble, November 25th 2013

08:30 Door opens

Session 1 : Chromatin and Signaling : an integrative plateform

Keynote by Geneviève Almouzni (Institut Curie, Paris)
Chromatin assembly and histone chaperones.

Valérie Borde (Institut Curie, Paris)
Role of the histone H3 lysine 4 methylation at the meiotic recombination hotspots in S. cerevisiae

Emilie Montellier (Institut Albert Bonniot, Grenoble)
Chromatin-to-nucleoprotamine transition is controlled by the histone variant TH2B during spermatogenesis

Coffee Break

Robert Schneider (IGMBC, Strasbourg)
Novel modifications – novel players in chromatin function

Johanna K. Samuelsson (Active Motif, California)
FFPE-ChIP-seq reveals the clinical importance of histone marks and their relation to DNA methylation in long-term stored FFPE colon cancer samples.

Lunch Break

Session 2 : A proteomics view of chromatin signaling

Michiel Vermeulen (Utrecht, Netherlands)
Reading the epigenome: developmental and evolutionary dynamics of 5-(hydroxy) methylcytosine readers in vertebrates

Mihaela Sardiu (Washburn lab, Stowers Institute, Kansas, USA)
A Proteomics approach for understanding protein networks underlining chromatin remodeling complexes

Cyril Boyault (Institut Albert Bonniot, Grenoble)
Understanding the protein network involved in heterochromatin formation by quantitative proteomics

Eric Trinquet (CisBio)
A new HTRF platform to investigate epigenetic targets

Coffee Break

Session 3 : A structural view of chromatin signaling

Keynote by Andreas Ladurner (University of Munich)
The chaperone FACT in complex with H2A-H2B and New tools for cell-type-specific genomics

Alessandro Tosi (University of Munich)
Structure and subunit topology of the INO80 chromatin remodeler and its interaction with the nucleosome

Daniel Panne (EMBL, Grenoble)
Structure of the p300 catalytic core and implications for HAT regulation and chromatin targeting

17:00 Closing remarks

Geneviève Almouzni

CHROMATIN ASSEMBLY AND HISTONE CHAPERONES

Laboratory of Nuclear Dynamics and Genome Plasticity, UMR 218 CNRS/Institut Curie, Research Center, 26 rue d'Ulm, F-75248 Paris cedex 05, France

Studies concerning the mechanism of DNA replication and repair have advanced our understanding of the stable transmission through multiple cell cycles of a faithful genetic material. Recent work has shed light on possible means to ensure the stable transmission of information beyond just DNA and the concept of epigenetic inheritance has emerged. Considering chromatin-based information, key candidates have arisen as epigenetic marks including DNA and histone modifications, histone variants, non-histone chromatin proteins, nuclear RNA as well as higher-order chromatin organization. Thus, understanding the dynamics and stability of these marks following disruptive events during replication and repair and throughout the cell cycle becomes of critical importance for the maintenance of any given chromatin state.
We will present our most recent work on this topic, considering mostly histone variant dynamics and histone chaperones during the cell-cycle and in response to DNA damage.

5 recent publications :

1. Filipescu D., Szenker E. & Almouzni G. (2013) Developmental roles of histone H3 variants and their chaperones. ***Trends Genet.,*** **29**, 630-640.
2. Adam S., Polo S.E. & Almouzni G. (2013) Transcription recovery after DNA damage requires chromatin priming by the H3.3 histone chaperone HIRA. ***Cell***, **155**, 94-106.
3. MacAlpine D.M. & Almouzni G. (2013) Chromatin assembly. In "DNA Replication", Cold Spring Harb. Perspect. Biol. Doi:10.1101/cshperspect.a010207, CSHL Press, pp. 197-218.
4. Abascal F., Corpet A., Gurard-Levin Z-A., Juan D., Ochsenbein F., Rico D., Valencia A. & Almouzni G. (2013) Subfunctionalization via adaptive evolution influenced by genomic context: the case of histone chaperones ASF1a and ASF1b. ***Mol. Biol. Evol.,*** **30,** 1853-1866. (Selected for F1000Prime)
5. Casanova M., Pasternak M., El Marjou F., Le Baccon P., Probst A.V. & Almouzni G. (2013) Heterochromatin reorganization during early mouse development requires a single-strand non-coding transcript. ***Cell Rep***. Sept 17. doi:pii: S2211-1247(13)00439-7. 10.1016/j. celrep.2013.08.015. [Epub ahead of print]

Valérie Borde

ROLE OF THE HISTONE H3 LYSINE 4 METHYLATION AT THE MEIOTIC RECOMBINATION HOTSPOTS IN S. CEREVISIAE

Laboratory of Nuclear Dynamics and Genome Plasticity, UMR 218 CNRS/Institut Curie, Research Center, 26 rue d'Ulm, F-75248 Paris cedex 05, France

During meiosis, homologous recombination is initiated by the formation of programmed double strand breaks (DSBs), which is essential for an accurate segregation of the homologs and the production of normal and viable gametes. Meiotic chromosomes are organized into arrays of loops that are anchored to the chromosome axis structure. Programmed DNA double-strand breaks (DSBs) that initiate meiotic recombination, catalyzed by Spo11 and accessory DSB proteins, form in loop sequences in promoters, whereas the DSB proteins are located on chromosome axes. Mechanisms bridging these two chromosomal regions for DSB formation have remained elusive. In budding yeast, DSBs occur mostly in promoters, in the nucleosome depleted region. In addition, the trimethylation of histone H3 lysine 4 (H3K4me3), a mark usually associated with active transcription, is enriched close to DSBs (Borde et al., 2009). Similarly, in the mouse, active recombination hot spots are enriched with H3K4me3 (Buard et al, 2009). We have shown that Spp1, a conserved member of the histone H3K4 methyltransferase Set1 complex, is required for normal levels of DSB formation and is associated with chromosome axes during meiosis, where it physically interacts with Mer2, an accessory DSB protein. The PHD finger module of Spp1, which reads H3K4 methylation close to promoters, promotes DSB formation by tethering these regions to chromosome axes and activating cleavage by the DSB proteins (Sommermeyer et al, 2013).
This study provides the molecular mechanism spatially linking DSB sequences to chromosome axes and explains why H3K4 methylation is important for meiotic recombination. This mechanism may be conserved in mammals where H3K4me3 is deposited at meiotic hotspots by the Prdm9 protein.

Emilie Montellier

CHROMATIN-TO-NUCLEOPROTAMINE TRANSITION IS CONTROLLED BY THE HISTONE VARIANT TH2B DURING SPERMATOGENESIS.

Montellier E, Boussouar F, Rousseaux S, Zhang K, Buchou T, Fenaille F, Shiota H, Debernardi A, Héry P, Curtet S, Jamshidikia M, Barral S, Holota H, Bergon A, Lopez F, Guardiola P, Pernet K, Imbert J, Petosa C, Tan M, Zhao Y,Gérard M, Khochbin S.

U823, Institut National de la Santé et de la Recherche Médicale INSERM, Institut Albert Bonniot, Université Joseph Fourier, Grenoble F-38700 France.

The most drastic chromatin remodelling occurs during spermatogenesis since almost all histones are removed and replaced by small sperm basic proteins, protamines. The conversion of male germ cell chromatin to a nucleoprotamine structure is fundamental to the life cycle, yet the underlying molecular details remain obscure. Here we show that an essential step is the genome-wide incorporation of TH2B, a histone H2B variant of hitherto unknown function.
The testis-specific H2B histone variant TH2B has been discovered about 40 years ago, however the physiological role of this variant remained unknown. We show that unlike other histone variants, TH2B replaces almost all canonical H2B during early stages of spermatogenesis. We used a mouse model in which TH2B is C-terminally modified and show that TH2B directs the final transformation of dissociating nucleosomes into protamine-packed structures. We used a mouse model depleted for TH2B and we show that absence of TH2B induces compensatory mechanisms that permit histone removal by up-regulating H2B and programming nucleosome instability through targeted histone modifications. Moreover we decribe that TH2B is also expressed during female gametogenesis and that TH2B reassembles onto the male genome during protamine-to-histone exchange after fertilization.
Thus, TH2B is a unique histone variant that plays a key role in the histone-to-protamine packing of the male genome and guides genome-wide chromatin transitions that both precede and follow transmission of the male genome to the egg.

Robert Schneider

NOVEL MODIFICATIONS – NOVEL PLAYERS IN CHROMATIN FUNCTION

Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS UMR 7104, INSERM U 964, Université de Strasbourg, 67404 Illkirch, France

Histone modifications are central to the regulation of all chromatin-based processes. Currently, the repertoire of known modifications is far from complete. We have a strong interest in unraveling the functions of novel sites as well as novel types of histone modifications.
So far most attention had been focused on modifications within the N-terminal tail of the histones. Whereas the function of histone tails as “signalling platforms” is more or less well established, the globular domains of histones and the nucleosomal core have long been regarded as simple structural scaffolds for nucleosome formation.
We identified modifications on the lateral surface of the histone octamer, at positions close to the DNA as key regulators of chromatin function. We showed that these modifications can act directly and can be causative for transcriptional activation. This is in contrast to e.g. histone tail acetylations and demonstrates that histone modifications are not simply by-products of other processes (e.g. transcription) but have a functional role in chromatin function. We therefore suggest that transcriptional regulators elicit their effects not only via signaling to histone-tails, but also via direct structural perturbation of nucleosomes by directing acetylation to the lateral octamer surface.

Johanna K. Samuelsson

FFPE-CHIP-SEQ REVEALS THE CLINICAL IMPORTANCE OF HISTONE MARKS AND THEIR RELATION TO DNA METHYLATION IN LONG-TERM STORED FFPE COLON CANCER SAMPLES.

Active Motif, Inc.

Colorectal cancer is the third most common cancer and the fourth leading cause of cancer-related deaths in the world. Genetic alterations (i.e. (mutations) are a hallmark of colon cancer, however recent advances in epigenetic research suggests that epigenetic alterations are potentially more frequent than genetic alterations. Changes in DNA methylation, as well as histone modifications, have been found in every tumor type studied to date, both benign and malignant, and have been associated with the progression of colorectal cancer. Thus, gaining further insight into these complex regulatory mechanisms is crucial to understanding disease susceptibility, initiation and progression

Chromatin Immunoprecipitation (ChIP) is a powerful tool for studying protein/DNA interactions. So far, ChIP studies have been limited mainly to cultured cells and nonhuman model systems. However, due to observed discrepancies between ChIP profiles in primary tissue and cell lines, identifying alterations in histone modification profiles directly in human tissue samples would be highly valuable. Formalin-fixed, paraffin-embedded (FFPE) samples represent the gold standard for storage of pathology samples and are an invaluable resource for biomedical research and retrospective analyses. Thus, we have developed an FFPE-ChIP protocol that generates interpretable and reproducible results detailing localization of active and repressive histone marks as well as transcription factors in normal and cancerous human colon FFPE samples, stored under less than optimal conditions for over 10 years. Further, genome wide comparison of H3K4me3 levels in normal and tumor human colon FFPE samples identified various expected cancer specific alterations. Correlating these ChIP results with DNA methylation and mutation data from the same samples provides the first multi-level epigenetic profiling of FFPE samples and highlights the value of performing ChIP in primary tissue samples.

Michiel Vermeulen

READING THE EPIGENOME: DEVELOPMENTAL AND EVOLUTIONARY DYNAMICS OF 5-(HYDROXY) METHYLCYTOSINE READERS IN VERTEBRATES

Ozren Bogdanović, Arne Smits, H. Irem Baymaz, Carmen Hidalgo, Felix Gnerlich, Thomas Carell, Ryan Lister, Jose Luis Gomez-Skarmeta, Michiel Vermeulen

Department of Molecular Cancer Research, University Medical Center Utrecht, The Netherlands

Methylation of cytosine residues at carbon atom 5 (5-meC) in higher eukaryotes represents a major mechanism via which cells can silence genes. Recent work from our laboratory revealed that 5-meC and its oxidative derivatives recruit a distinct and highly dynamic set of 'readers' during stem cell differentiation into a neuronal lineage. This implies that the functional readout of DNA (hydroxy)methylation may be affected by the presence or absence of certain readers during development. To further address this, we made use of the model organism zebrafish (Danio rerio). Using transgenic approaches, we demonstrate that 5-meC displays reduced repression potential in early zebrafish embryos compared to later stage embryos. Next, we applied quantitative mass spectrometry-based proteomics to identify readers for 5-meC in nuclear extracts from early and late stage zebrafish embryos. We observed strong inter-species conservation in protein families that associate with 5-meC and 5-hmC during embryogenesis. Zinc finger proteins are predominantly interacting with 5-meC during early embryonic stages, whereas differentiation factors such as Hox proteins bind to 5-meC during organogenesis. 5-hmC recruits transcriptional regulators and DNA repair proteins, both in (differentiated) mouse ES cells and zebrafish embryos. These observations provide evidence that the DNA damage response has an evolutionary conserved role in active DNA demethylation, despite the different timing of DNA methylation remodeling that is observed between mammals and lower vertebrates. We identified the Foxk family of proteins as constitutive 5-meC readers and we demonstrate that their depletion perturbs normal development. Finally, we observed that MBD2 does not interact with 5-meC in early stage embryo's or mouse embryonic stem cells, whereas this protein is a prominent 5-meC reader in later stage embryos and neuronal progenitor cells. Strikingly, overexpression of MBD2 in early stage zebrafish embryos efficiently restores 5-meC mediated transcriptional repression. Overall, these data indicate that a diverse and dynamic set of proteins 'read' DNA methylation during early vertebrate development and that the transcriptional repressive potential of DNA methylation is highly affected by the cellular complement of expressed/bound 5-meC readers.

Mihaela E. Sardiu

A PROTEOMICS APPROACH FOR UNDERSTANDING PROTEIN NETWORKS UNDERLINING CHROMATIN REMODELING COMPLEXES

Mihaela E. Sardiu and Michael P. Washburn

Stowers Institute for Medical Research, Kansas City, MO 64110

Protein complexes are major building blocks of biological systems. Therefore, when aiming to understand biological events on a systems level, it is essential to identify and characterize protein complexes through parameter quantification, followed by bioinformatics analysis and mathematical modeling. Therefore, we tested the possibility of using quantitative proteomics data in characterizing dynamic protein complexes. Using data sets generated from protein complexes involved in chromatin remodeling and transcription, we could demonstrate and experimentally verify that quantitative proteomics can be used for generating probabilistic protein networks. However, since many protein complexes are very stable or very complex, the value of this approach is limited. Therefore we developed approaches to overcome this problem using perturbations in order to understand how proteins interact and are spatially arranged to form these macromolecular complexes. By using a combination of computational approaches and quantitative proteomics data generated from yeast (SAGA and INO80) and human (Sin3/HDAC) complexes we could provide novel insights into the dynamic changes and associations of proteins in these chromatin networks.

Cyril Boyault

UNDERSTANDING THE PROTEIN NETWORK INVOLVED IN HETEROCHROMATIN FORMATION BY QUANTITATIVE PROTEOMICS.

Boyault C, Gilquin B, Cattaneo M, Perazza D, Emadali A, Couté Y, Verdel A.

INSERM/UJF Unité 823, Institut Albert Bonniot, Grenoble, France

Over the last decade, Schizosaccharomyces pombe emerged as a model organism to study heterochromatin, a chromatin state that imposes an epigenetic transcriptional silencing. In this organism, the recruitment to chromatin of a trimeric complex called "RITS" (RNA Induced Transcriptional Silencing complex) is sufficient for heterochromatin formation. Despite a multiplicity of studies defining the mechanisms involved in this process, the identity of RITS interactors and their roles remain largely unknown. To understand how RITS mediates heterochromatin formation, we aimed at improving our knowledge of the protein network contributing to RITS function. We combined mass spectrometry analyses based on label-free quantitative proteomics to classical approaches of microscopy, gene expression analysis and yeast genetics. This strategy identified RITS interactome and shows the relative contribution of the 3 RITS sub-units to this interactome. In addition, it suggests a molecular link between RITS and various machineries such as DNA replication and repair ones.

Eric Trinquet

A NEW HTRF PLATFORM TO INVESTIGATE EPIGENETIC TARGETS

VP Product Development, Cisbio Bioassays, Codolet, France

Epigenetic mechanisms are inheritable factors, like DNA methylation or histone modification, which regulate gene expression without modifying the DNA sequence. In the past years, different classes of histone modifying enzymes as well as protein complexes which selectively bind modified histones have been demonstrated to have strong disease association in cancer, mental disorders, autoimmune disease or diabetes. To facilitate the identification and the characterization of compounds that can modulate their activity, Cisbio Bioassays has established a new EPIgeneous™ platform based on its proprietary homogeneous fluorescence assay technology, HTRF. This platform gathers today a selection of assays that allow the study of methyl transferases or demethylases in combination with a large variety of substrates, including nucleosomes, or in a cell-based context. In addition, powerful and generic detection solutions have been established to investigate interactions between modified histones and reader domains. Due to their robustness and their miniaturization, EPIgeneous™ assays are fully compatible with high-throughput screening campaigns, as well as for global investigation of epigenetic targets and related compound optimization.

Andreas Ladurner

THE CHAPERONE FACT IN COMPLEX WITH H2A-H2B AND NEW TOOLS FOR CELL-TYPE-SPECIFIC GENOMICS

Department of Physiological Chemistry and Centre for Integrated Protein Science Munich (CIPSM), Butenandt Institute, Ludwig Maximilians University of Munich, Butenandtstrasse 5, 81377 Munich, Germany.

Alessandro Tosi

STRUCTURE AND SUBUNIT TOPOLOGY OF THE INO80 CHROMATIN REMODELER AND ITS INTERACTION WITH THE NUCLEOSOME

Alessandro Tosi, Caroline Haas, Franz Herzog, Andrea Gilmozzi, Otto Berninghausen, Charlotte Ungewickell, Christian B. Gerhold, Kristina Lakomek, Ruedi Aebersold, Roland Beckmann and Karl-Peter Hopfner

Department of Biochemistry and Gene Center, Ludwig-Maximilians-University, Munich, Germany

The family of Swi2/Snf2 enzymes comprises complex multiprotein assemblies that remodel DNA:protein complexes. INO80, a member of the INO80/SWR1 family of chromatin remodelers belongs to the Swi2/Snf2 ATPases and is a large 1.3MDa complex composed of 15 different subunits. INO80 has important roles in replication, transcription and DNA repair and is required to maintain genome integrity, yet little is known about its structure and mechanism. The INO80complex uses the energy of ATP to remodel chromatin by modifying its structure and composition, for example exchanging canonical histone H2A with the variant H2AZ. Here, we report the structure of INO80 determined by a hybrid approach of electron microscopy, chemical cross-linking and mass spectrometry and biochemical analysis. The elongated and flexible 3Dstructure of INO80 is markedly different to other huge chromatin remodelers (SWI/SNF and RSC). Cross-linking and mass spectrometric analysis indicated a modular organization within the INO80 subunits and unraveled the interaction network of all subunits with domain resolution. Furthermore, we could assign a Rvb1/2 hetero-dodecamer in contact with the Swi2/Snf2 domain to the cryo-EM structure and could map the other INO80 modules. The interaction map of an INO80-nucleosome complex and its structural flexibility suggest howINO80 binds a nucleosome. Our analysis establishes a first structural framework for a large chromatin remodeler and provides insights into the remodeling mechanism of INO80.

Daniel Panne

STRUCTURE OF THE P300 CATALYTIC CORE AND IMPLICATIONS FOR HAT REGULATION AND CHROMATIN TARGETING

EMBL Grenoble, 6 Rue Jules Horowitz, Grenoble 38042, France

Gene regulation in higher eukaryotes requires recruitment of the transcriptional co-activators CBP/p300 that associate with transcriptional regulators and integrate a large number of signal transduction pathways. Recruitment of CBP/p300 results in acetylation and remodeling of inhibitory chromatin. CBP/p300 contain both chromatin interaction (Bromo and PHD) and modification (HAT) domains. It has been hypothesized that co-occurrence of such 'reader' domains is required for multivalent recognition of appropriately modified chromatin but how chromatin recognition and modification are coupled is currently unknown. To understand the role of the tandem Bromo and PHD domains in chromatin substrate targeting and regulation of p300 activity we have crystallized the entire catalytic 'core' of p300 including the Bromo, PHD and HAT domains. The structure reveals that the Bromo, PHD and HAT domains form an assembled conformation in which the Bromo- and PHD-domains rigidly dock against the HAT domain. A previously unsuspected RING domain is inserted into the non-continuous PHD domain. This RING domain is flexibly attached to the HAT domain and partially covers its substrate binding site. We found that the RING domain has an autoinhibitory function for p300 activity and that mutations that destabilize RING attachment lead to upregulation of HAT activity. Such mutations are found in different diseases including breast cancer, lymphoid neoplasms and Rubinstein-Taybi Syndrome. Upregulation of CBP/p300 HAT activity provides a novel paradigm for the dysregulation of these key epigenetic regulators and makes them attractive pharmacological targets. Detailed investigation of chromatin substrate recognition showed that the Bromodomain preferentially interacts with histones containing combinations of acetylations rather than singly modified sequences, whereas the p300 PHD domain did not interact with canonical substrates. Our results demonstrate that the Bromodomain substrate specificity is compatible with HAT substrate acetylation patterns suggesting that positive feedback is likely an important component in establishment of active chromatin states.