Understanding chromatin and chromosomes: from static views to dynamic thinking

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The 106th Boehringer Ingelheim Fonds International Titisee Conference, Reconstituting chromatin: from self-assembly to self-organization, took place in October 2012. The organizers, Andrea Musacchio and Tom Muir, brought together biologists, chemists and physicists to discuss the principles of chromosome assembly and organization. Topics of discussion ranged from novel insights gained from the static views provided by crystal structures to analyses of chromatin dynamics inside living cells.

The long DNA molecules that constitute each chromosome within the nucleus of eukaryotic cells wrap tightly around octameric assemblies of histone proteins to form chains of nucleosomes. This organization into chromatin regulates all major aspects of chromosome metabolism, from gene expression, DNA replication, damage repair and recombination, to segregation. Packaging into nucleosomes is only the first step in the complex three-dimensional arrangement of a cell's genome. Despite considerable advances during the past few years, the nature of higher order chromatin structures remains poorly understood. Moreover, nucleosomes come in many different flavors. This diversity stems not only from variants of the core histones but also from a range of posttranslational histone *modifications* whose functional significance, alone or in combination, remains largely mysterious. The 106th Boehringer Ingelheim Fonds International Titisee Conference focused on current approaches that explore the basic organizational and regulatory principles of chromatin and its reconstitution in vitro. In this report, we briefly highlight some of the main topics of discussion.

Nucleosome positioning

What makes nucleosomes go to the right position on a DNA helix at the right time is a fundamental puzzle in the chromatin field. Early *in vitro* reconstitution studies suggested that the sequence of the DNA double helix is the main determinant of nucleosome positioning, and

major attempts have therefore been undertaken to predict the location of nucleosomes using *in silico* methods. John van Noort (Leiden U., The Netherlands) presented a thermodynamic model that is able to accurately reproduce not only the positioning of nucleosomes on defined short DNA templates at high resolution, but also genome-wide nucleosome occupancy maps based on the periodicity of four dinucleotide sequence motifs. Song Tan (Penn State U., USA) pointed out that thymidine-adenine base steps might be particularly important in this respect, because they are thought to allow for an unusual structural flexibility and hence could stabilize the interactions between the DNA double helix and histones H3 and H4. Philipp Korber (LMU, Munich, Germany) argued that in addition to the intrinsic DNA sequence component, nucleosome positioning *in vivo* is largely controlled by extrinsic factors such as chromatin remodelling enzymes. The information gained from predictions, in combination with novel approaches to map nucleosome positions *in vivo* with single base-pair precision, may be crucial to allow the reconstruction of chromatin substrates incorporating specific promoter sequences, a goal that was put forward by Tim Richmond (ETH, Zurich, Switzerland).

The assembly of histone proteins into nucleosomes requires the action of histone chaperones and ATP-driven motor proteins such as CHD1, which is a SNF2-type chromatin remodeling factor required for the incorporation of the histone H3 variant H3.3 into nucleosomes. Alexandra Lusser (Innsbruck Medical U., Austria) presented mechanistic insights into the coupling between the ATPase activity and nucleosome assembly function of CHD1. Moreover, little is known about the dynamics of nucleosome assembly. James Kadonaga (U. California San Diego, USA) discussed a two-step model for nucleosome formation. According to this model, histone-chaperone complexes first deposit histones onto DNA to form 'pre-nucleosomes'. Pre-nucleosomes appear similar to canonical nucleosomes by atomic force microscopy, but wrap merely half of the length of DNA around them. In the second step, they are converted into *bona fide* nucleosomes by the action of ATP-dependent motor proteins such as ACF. While the formation of pre-nucleosomes is thought to occur on a timescale of seconds, the conversion of pre-nucleosomes into canonical nucleosomes takes several minutes. One of the challenges ahead is finding a way to distinguish between pre-nucleosomes and mature nucleosomes *in vivo*. Another one is to identify the contact points between DNA and histones in the pre-nucleosome. A related key question is whether the spacing between individual nucleosomes is already determined at the time of their deposition, or whether nucleosomes first assemble at more or less random locations and are then moved to their final positions. Evidence for the latter possibility comes from the analysis of mutant versions of CHD1.

Specialized nucleosome structures exist at specific chromosomal loci such as centromeres, where the canonical H3 histone is replaced by CENP-A (also known as CenH3). These CENP-A nucleosomes act as building platforms for the assembly of kinetochores, which in turn mediate the interaction between chromosomes and spindle microtubules to empower chromosome movement during cell division. The mechanisms that dictate exclusive assembly of CENP-A nucleosomes at centromeres are still largely unresolved. Robin Allshire (U. Edinburgh, UK) reported that, in fission yeast, incorporation of CENP-A nucleosomes at centromeric regions depends on several factors. One factor is the DNA sequence of the central domain, which is different for each of the three fission yeast centromeres. Another factor is the establishment of heterochromatin regions flanking the central domain. In addition, low levels of transcription of the central domain are required. One intriguing possibility is that active transcription destabilizes H3-containing nucleosomes and favours CENP-A incorporation in the wake of the passing RNA polymerase.

Understanding the mechanisms that balance the competition of H3 and CENP-A chromatin assembly at the correct chromosomal addresses will strongly benefit from insights into the biophysical properties of the different nucleosome variants. Ben Black (U. Pennsylvania, USA) presented hydrogen/deuterium exchange (H/DX) studies that show that, despite a

rigidified core, CENP-A nucleosomes have loosened terminal DNA contacts. Thus, CENP-A nucleosomes might unwrap the ends of nucleosomal DNA more readily. This notion could probably be best tested by methods such as the combined single-molecule fluorescence and force spectroscopy approach presented by Taekjip Ha (HHMI and U. Illinois, Urbana-Champaign, USA), which measures the forces that are required to unwrap DNA helices from histone octamers.

Higher order structure

While recent years have seen a tremendous increase in knowledge about the assembly of DNA helices into nucleosome fibres, our understanding of how these fibres are arranged in the context of a complete chromosome is still limited. At least *in vitro*, tandem arrays of nucleosome positioning sequences can fold into regular filaments of ~30 nm diameter. Whether such ordered conformations also exist inside the cell nucleus is, however, a matter of intense discussion. An important next step might be to test whether higher-order chromatin structures can be reconstituted with natural DNA sequences, for example using specific promoter sequences mentioned above (Richmond). In addition to structural studies, approaches that assess the dynamic biophysical properties of nucleosome arrays might help to elucidate whether and how individual nucleosomes can interact to form the next level of organization. Quantitative measurements of the stacking forces between nucleosomes, which can be deduced from pulling on 30 nm nucleosome arrays using magnetic tweezers (van Noort), might contribute to refine models of possible higher order assemblies.

To test whether the characteristics of chromatin fibres determined by *in vitro* measurements are relevant in the context of a cell, approaches are needed that assess the mechanical properties of chromosomes *in vivo*. Kevin Chalut (Cavendish Laboratory, U. Cambridge, UK) introduced a microfluidics system that can be used to optically stretch single cells and to deduce physical attributes of chromatin inside the nucleus. This setup is able to detect changes in stiffness when chromatin is either compacted by Mg^{2+} addition or expanded by inhibition of histone deacetylase activity, suggesting that it can be applied to follow, for example, the changes in chromatin architecture as cells differentiate.

In addition to novel experimental approaches, computational modelling will have an important impact on understanding higher order chromatin arrangements. One approach to approximate the dynamics of chromatin is the use of worm-like chain models that are based on data of the physical properties of chromatin fibres measured *in vitro* (Ha). Moreover, the increasing amount of data that describe the conformation of chromosomes in nuclei using massive parallel sequencing approaches (e.g. 5C or HiC) will help to generate models that describe the folding principles behind chromatin filaments; a technique that was discussed by Diego di Bernardo (TIGEM, Naples, Italy).

An increasing body of evidence suggests that the global organization of chromatin fibres requires the function of additional chromosomal components, such as the multi-subunit cohesin protein complex. Cohesin is best known for its role in holding sister chromatids together to allow their bi-orientation on the mitotic spindle at the time of cell division. In addition to its mitotic role, cohesin contributes to the spatiotemporal regulation of gene expression through a poorly understood mechanism that is thought to involve stabilization of chromatin loops, as discussed by Ana Losada (CNIO, Madrid, Spain). The residence time of cohesin on chromatin is regulated by its interaction with a protein named Wapl. Jan-Michael Peters (IMP, Vienna, Austria) reported that depletion of this factor in mouse cells causes an intriguing increase in the compaction of interphase chromosomes. How these changes in structural organization affect gene expression is currently under investigation.

Chromosomes undergo remarkable conformational changes upon entry into M phase, resulting in the formation of rod-shaped pairs of sister chromatids. While it is not yet understood how this additional level of structural organization is achieved, the cohesin-related condensin complex and topoisomerase II have been identified as key players in this chromosome condensation process. Tatsuya Hirano (RIKEN, Japan) presented exciting progress towards the reconstitution

of mitotic chromosomes *in vitro*, using simple chromatin templates and purified proteins, including condensin and topoisomerase II. This approach will probably be a key step towards unravelling the molecular mechanisms behind the functions of condensin and topoisomerase in mitotic chromosome formation, and should provide insights into the general concepts of higher order levels of chromatin organization.

Recognizing nucleosomes

How are chromatin structures identified by the proteins that bind to them? Answers to this question come from recent co-crystal structures of protein-histone complexes—e.g. between the chaperone Daxx and a dimer of histones H3.3/H4, presented by Simon Elsässer (MRC-LMB, Cambridge, UK)—or from electron microscopy reconstruction of nucleosomes bound to enzymes such as the chromatin remodeller ISWI (Richmond). One of the challenges for future structural work will be the elucidation of even larger assemblies, for example complexes formed between nucleosomes and Ran–RCC1 heterodimers or the histone acetyl transferase NuA4 (Tan). High-resolution structures will presumably also provide pivotal insights into the regulatory roles of posttranslational modifications, either of the nucleosome core subunits or of their binding partners—e.g. the acetylation of the Sir3 BAH domain presented by Fabrizio Martino (MRC-LMB, Cambridge, UK).

In addition to structural studies, novel biochemical assays to assess the interaction between proteins and nucleosome fibres will help explain how enzymes can specifically act in a chromatin context. One such example is the enzyme PARP-1, which associates with chromosomes and attaches chains of poly(ADP-ribose) to histones and other target proteins upon DNA damage. Insights into how PARP-1 recognizes nucleosome/linker DNA complexes came from novel quantitative gelshift and solution assays using fluorescently labelled PARP-1, presented by Karolin Luger (HHMI and Colorado State U., Fort Collins, USA). The results from these assays suggest that, upon auto-PARylation, PARP-1 functionally switches from a chromatin binding protein to a nucleosome assembly factor. While these and similar approaches can now be used to determine precise affinities of proteins to nucleosome fibres, creating nucleosome arrays with specific posttranslational histone modification patterns has remained a difficult task. Expressed protein ligation provides a chemical biology technique to overcome this limitation. Tom Muir (Princeton U., USA) discussed the development of a tool set for the custom design of modified nucleosome arrays, which can then, for example, be used in combination with quantitative proteomics techniques to identify proteins that recognize a particular 'histone code'. Eventually, it may be possible to create libraries of barcoded mononucleosomes, each containing a different modification, to assemble specific arrays for high throughput biochemistry applications.

One of the most intriguing macromolecular assemblies that bind to chromosomes is the kinetochore. Aaron Straight (Stanford U., USA) reported on the progress of assembling a functional kinetochore by incubating chromatin templates made from recombinant CENP-A nucleosomes in *Xenopus* egg extracts, which might one day enable recapitulating chromosome segregation in the test tube. Reconstituting kinetochore complexes will most likely provide crucial insights into how they link centromeric chromatin to microtubules, sense correct spindle attachments and destabilize incorrect ones. The Knl1/Mis12/Ndc80 (KMN) kinetochore subcomplex is essential for integrating these different functions and thereby acts as the CPU of the kinetochore. Andrea Musacchio (MPI Dortmund, Germany) presented an intriguing model for the assembly of the KMN subcomplex on a Constitutive Centromere Associated Network (CCAN) platform. Additional insights into the building principles behind kinetochores may also come from the study of species that are evolutionary distant to traditional model organisms and that are now becoming accessible through the advancement of genomic sequencing tools. One such species is *Trypanosoma brucei*, which surprisingly lacks CENP-A. Bungo Akiyoshi (U. Oxford, UK) has already identified several kinetochore proteins in this organism, none of which

show recognizable homology to known kinetochore components.

Conclusion

We would like to end this report by thanking both organizers, Andrea Musacchio and Tom Muir, for the 'software' side of this meeting, but also Boehringer Ingelheim Fonds for providing the superior 'hardware'.

This conference has opened our minds to classic and novel technologies and ideas to continue exploring the ever-changing field of chromatin and chromosome dynamics.

Conflicts of Interests

The authors declare that they have no conflict of interest.