

From Beads on a String to the Pearls of Regulation: the Structure and Dynamics of Chromatin

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From Beads on a String to the Pearls of Regulation: the Structure and Dynamics of Chromatin

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Abstract

The assembly of eukaryotic chromatin, and the bearing of its structural organization on the regulation of gene expression, were the central topics of a recent conference organized jointly by the Biochemical Society and Wellcome Trust. A range of talks and poster presentations covered topical aspects of this research field and illuminated recent advances in our understanding of the structure and function of chromatin. The two-day meeting had stimulating presentations complemented with lively discourse and interactions of participants. In the present paper, we summarize the topics presented at the meeting, in particular highlighting subjects that are reviewed in more detail within this issue of *Biochemical Society Transactions*. The reports bring to life the truly fascinating molecular and structural biology of chromatin.

Introduction

Eukaryotic chromatin has been the subject of extensive biochemical studies for nearly four decades. These efforts have culminated in the crystal structure of the fundamental unit of chromatin, the nucleosome, and have established roles for covalent modifications of histones as signals for gene regulation, epigenetic effects, and they continue to provide us with understanding of other biological processes as shown most recently by studies of the role of chromatin in the response to DNA damage [1]. Interpretation of the information encoded through the covalent modifications of chromatin involves the co-operation of numerous recognition modules, and the downstream effects of these signals are likely to be dependent on structural context. The organization of nucleosomes along the DNA and their

higher-order assembly also affects gene function [2,3]. These structure–function relationships in chromatin were the theme of a conference organized jointly by the Biochemical Society and the Wellcome Trust, which took place at the Wellcome Trust Genome Campus at Hinxton Hall near Cambridge in August 2011.

The meeting brought together over 100 researchers and an important aim of catalysing exchange of ideas that help to develop future activities in the field. Details about the topics discussed by the speakers at the meeting are provided below. In addition to these talks, numerous posters were presented, with their high quality demonstrating the excellent science being undertaken within this research field. Abstracts submitted for all presentations and posters are hosted on the web pages for the meeting (http://www.biochemistry.org/meetings/programme.cfm?Meeting_No=SA122).

The meeting was held in honour of Dame Jean Thomas, to celebrate her substantial contributions to the chromatin field, as well as her roles as an inspiring mentor and teacher, and to recognize her tremendous services to the scientific

Key words: chromatin structure, DNA repair, nucleosome positioning, nucleosome structure, protein–DNA interaction.

Abbreviations used: Asf1, anti-silencing function 1; CTCF, CCCTC-binding factor; HMG, high-mobility group; ISW1, imitation switch chromatin remodelling 1; RCC1, regulator of chromosomal condensation 1.

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community through her activities within numerous scientific organizations.

The meeting brought together many of Jean's past colleagues, although one person in particular, her colleague and dear friend Jonathan Widom, was missed greatly. As many readers may be aware, Jonathan passed away unexpectedly a week before the meeting. In a celebration of his scientific contributions, Jean gave a moving tribute to Jonathan, describing his achievements and personal influence on the field. Jean first met Jonathan nearly 30 years ago when he was a postdoctoral researcher at the MRC Laboratory of Molecular Biology, where he worked with Aaron Klug to study chromatin filament structure. Well known for his penetrating intellect and rigorously logical approach to solving difficult problems, Jonathan had a deep understanding of biophysical chemistry, which he applied through physical techniques to address key biological problems. His precise and rigorous thinking sometimes made him appear intense and formal, and yet he exuded personal warmth, deep humanity and compassion. He was well regarded as a great mentor, who was encouraging and supportive to his younger colleagues. Many of the participants were familiar with Jonathan's work on preferred sequence periodicities in nucleosome positioning and, although not everyone will agree with the interpretation of these data, there was stimulating and engaging debate of this issue throughout the meeting, in a scientific spirit that Jonathan would have approved of and enjoyed.

Higher-order structure of nucleosomes

Eukaryotic DNA is packaged in nucleosomes, and the spacing of nucleosomes to form regular arrays is important for efficient packaging into a compact chromatin fibre. The relative importance of DNA sequence preference and chromatin remodelling factors in defining the nucleosome positions is still a matter of much debate in the field. The presence of a particular DNA sequence within a nucleosome or in the linker region also affects its potential to interact with DNA-binding proteins. The first session of the meeting focused on the functional implications of nucleosomal positioning in the genome.

Sarah Teichmann, the 2011 Colworth Medal awardee, described correlations of transcription-factor-binding sites and nucleosome positions in yeast and provided evidence that transcription factor-binding sites associated with gene activation occurred preferentially within nucleosomes, whereas binding sites associated with repression occurred within linker regions [4]. She showed further that binding of transcription factors resulted in remodelling of nucleosome positioning, favouring the idea that DNA sequence preference is the important driver of nucleosome positioning. Similar correlations were examined in differentiating leucocytes, which is a good experimental system for its tractability as a single-step cellular differentiation process. Andrew Travers [5] continued with this theme and described some of the results from the Widom laboratory, and provided a broad and insightful overview of the data supporting sequence

periodicities for preferred nucleosome-binding positions, from the original study on chicken erythrocyte chromatin by Sandra Satchwell et al. [6] 25 years ago.

The nucleus is replete with abundant histones that are not part of the nucleosome particle, the so-called 'linker' histones, and which appear to play a role in chromatin higher-order assembly and repressive structures. There are also highly basic non-histone proteins, the HMG (high-mobility group) proteins. Jean Thomas [7] described the structure and function of these proteins, the interplay between their two domains and the role of the natively unstructured tail in favouring a closed state, and the role of HMG proteins as catalysts for recruiting transcription factors.

Chromatin dynamics

Nucleosomes are rarely at rest, but are in a state of flux along the DNA, and one type of machine that modulates this dynamic behaviour are the remodelling factors. Tim Richmond [8] described the structure of the yeast ISW1 (imitation switch chromatin remodelling 1) chromatin remodelling factor and provided structural data for the interaction of ISW1 on a unit of two nucleosome particles and showed how ISW1 could set the spacing between adjacent nucleosomes. The data from the Richmond laboratory provide support for ATP-dependent remodelling factors as the important driver of nucleosome positioning.

The location of nucleosomes on genomic DNA is affected by active transcription and replication. Ozgen Deniz [9] described the relocation of nucleosomes during the yeast cell cycle, which were correlated with cell-cycle-regulated genes, centromeres and replication origins. The data presented showed a very specific pattern of nucleosome positioning around the transcription start site of genes, which was proposed to result from the physical properties and relative stiffness of the DNA in these regions. In a related study, but with contrasting conclusions, Philipp Korber [10] presented some striking data on the location of nucleosomes on DNA reconstituted *in vitro*. The *in vitro* constituted nucleosomes did not show the same positions as those *in vivo*, but required the addition of ATP-dependent remodelling factors to replicate the nucleosome positioning seen *in vivo*.

Telomeres, the termini of eukaryotic chromosomes, have been a puzzle for their unusual structures that must be maintained by a dedicated polymerase: the reverse transcriptase telomerase. Daniela Rhodes [11] presented the structure of human telomerase, based on cryo-electron microscopy with a resolution sufficient to show that the protein is a dimer, and the NMR structure of the ADD domain of the ATRX (α -thalassaemia/mental retardation syndrome X-linked) factor.

Chromatin has a convoluted structure within the densely packed nucleus, but data from cross-linking experiments suggest that defined genes may be compartmentalized in clusters of co-ordinated activity. Kai Kruse [12] spoke about the analyses of cross-linking data and discussed correlation for co-ordinated transcriptional activity in these clusters.

An interesting hypothesis was proposed that there may be organizing centres that maintain the genomic architecture.

Modification of chromatin

The surface of the nucleosome is a target for recognition by other proteins. One of these is the RCC1 (regulator of chromosomal condensation 1) factor, required to create the Ran-GTP gradient for spindle body homing. Song Tan [13] described the high-resolution crystal structure of the complex between RCC1 and the nucleosome, and also gave an insightful account for how his research group developed models for the complex in advance of the structure determination. Recognition of histones by chaperones was also the focus of Mair Churchill [14], who described the crystal structure and proposed mechanism of the Asf1 (anti-silencing function 1) protein in blocking the formation of H3/H4 heterotetramers and loading H3/H4 dimers on to DNA.

Jochen Wuerges [15] described structural studies of Arp8 subunit of the chromatin remodelling Ino80 assembly. The protein is an actin homologue, and the crystal structure can rationalize why the protein, unlike its actin cousin, does not oligomerize, although it binds GTP.

Tom Miller [16], from the MRC Laboratory of Molecular Biology in Cambridge, won the Biochemical Society Centenary poster prize at the meeting. Within a selected oral presentation, he described a chemical fragment library for modulation of the PHD (plant homeodomain) finger and highlighted the possibility of modulating small protein module interactions allosterically, which is an interesting topic for rational drug design of chromatin-binding targets.

Effects of chromatin on transcription

Signalling pathways at the membrane eventually have an impact on transcription, and the downstream targets of the signalling can include histone modification assemblies. Alena Krejčí [17] (recipient of a Biochemical Society Early Career Research Award), addressed the specificity in the pathway of cell signalling, converging on transcription control and influence of co-operation of transcription factors on pathway specificity.

How binding sites for transcription factors evolve over time is an interesting question, although it is difficult to assess owing to the short nature of most transcription factor-binding sites. Dominic Schmidt [18] discussed the binding of the transcription factor CTCF (CCCTC-binding factor), which recognizes a relatively long DNA sequence, across a range of mammalian lineages. He provided evidence that new CTCF sites were generated during evolution via repeat driven expansions.

Chromatin influences the response to DNA damage

The final session included topics related to chromatin indirectly: DNA damage repair and the response induced by such damage. Steve Jackson [19] provided an overview

of the mechanism of the DNA damage response and characterization of the factors that are involved in recognition of double-stranded DNA breaks, involving rapid and co-operative assembly of the repair machinery at the site of damage. ATP-dependent remodelling complexes are implicated in the response to DNA damage, and Jessica Downs [20] described studies on the yeast RSC (remodelling the structure of chromatin) complex. Evidence was presented that this complex is recruited to sites of DNA double-strand breaks and that it remodels nucleosome positions, which helps with resection, signalling and repair. Chromatin remodelling around promoter sites was also the subject of the final talk by Tom Owen-Hughes [21], using high-throughput sequencing of yeast strains to align transcription start sites and nucleosome positions. The results indicate that ISW1- and Chd1-containing remodelling enzymes maintain positioned nucleosomes at transcription start sites.

Summary

Four decades of biochemical and molecular studies of eukaryotic chromatin have identified mechanisms by which its structure influences its biology. Much has been learned during this time, but, as indicated during this meeting, there are many opening avenues to explore as well as some debates that continue to evade a single widely accepted solution. New data are identifying unexpected mechanisms by which chromatin regulates gene expression, bringing together diverse areas of research that range from epigenetics to how cells respond to cellular stress, such as DNA damage. It is clear that a complete interpretation of the biology requires a deep understanding of the structural context of chromatin.

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