Nuclear visions enhanced: chromatin structure, organization and dynamics

Eran Meshorer, Harald Herrmann & Ivan Raška

The EMBO Workshop on 'Chromatin Structure, Organization and Dynamics' took place in April 2011 in Prague, Czech Republic. Scientists from the life sciences, chemistry and biophysics presented their latest data on the generation of three-dimensional and, eventually, four-dimensional models of the genome, working to correlate changes in the organization of chromatin with the functional state of the genome.

Ten years ago, the 'champion of chromatin' Alan P. Wolffe, was tragically killed in a road-traffic accident in Rio de Janeiro, Brazil. In one of his last review papers, he emphasized the view that the conformational instability of nuclear structure is essential for the functional flexibility of the nucleus. At the time, it seemed that each week brought a new study on the dynamic exchange of nuclear components, as followed by 'frapping' of GFP-tagged proteins. These 'illuminations' or 'visions' of living cells triggered new insights and questions about the molecular nature of chromatin. Wolffe became convinced that the complement of nuclear components is in a dynamic state, and that this will eventually "facilitate a continual read-out of a particular metabolic or cell-cycle state" of the respective cell (Wolffe & Hansen, 2001).

At Charles University in Prague, Czech Republic, the oldest university in middle-Europe, medical research has been conducted for more than 600 years. The EMBO workshop on 'Chromatin Structure, Organization and Dynamics', held in April 2011, was organized by Ivan Raška (Charles U., Prague), Roland Foisner (Medical U. Vienna, Austria) and Yosef Gruenbaum (The Hebrew U. of Jerusalem, Israel), and documented the impressive speed at which scientific discovery rushes on. The term chromatin was first coined and used by Walther Flemming and colleagues, as a technical term, towards the end of the nineteenth century.

What does it mean today? The Chromatin Database (www.chromdb.org) suggests that, "Today, the word chromatin is mostly used by molecular geneticists to describe DNA associated with any of the numerous proteins that help organize, activate or repress DNA". As the meeting demonstrated, the time is ripe for the generation of three-dimensional (3D) and, eventually, four-dimensional models of the genome, in order to correlate changes in the organization of chromatin with the functional state of the genome. The participants were shown a variety of results generated with new methods including single-molecule methods, powerful molecular analyses—such as Pulse-SILAC mass spectrometry—and new imaging procedures including cryo-electron tomography and light-optical nanoscopy. These were matched by impressive results from 'classical' experimental techniques such as X-ray crystallography.

Nuclear organization

The idea that interphase chromosomes are organized in the form of chromosome territories has received wide support in the last ten years. Thomas Cremer (Ludwig-Maximilians U., Munich, Germany) attempts nothing less than dynamic 'cartography' of the nucleus. He reported that chromatin movements are fast in embryonic stem cells, but slow in fibroblasts, suggesting that such intricate details of the living nucleus are different depending on the cell type being observed, reflecting fundamentally different states of nuclear activity in these cells. Accordingly, the complement of factors that are engaged in the generation of the 3D architecture of the nucleus by anchoring portions of interphase chromosomes to the nuclear envelope—that is, nuclear envelope transmembrane proteins (NETs)—is probably more complex than was anticipated a few years ago. Eric Schirmer (U. Edinburgh, UK) reported the results of a screen conducted in his lab for NETs that reposition chromosomes. His group found that several tissue-specific NETs can recruit distinct subsets of chromosomes to the nuclear periphery. The molecular mechanism and the additional factors that the NETs need to carry out their job remain elusive, but they seem to be principal regulators of chromosome territories and nuclear architecture.

The regulation of chromatin organization within the nucleus was further addressed

by Susan Gasser (Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland). Her group has explored the role of promoter-bound factors for the positioning of developmentally regulated genes within the nucleus, and the mechanisms that sequester facultative heterochromatin during *Caenorhabditis elegans* development. She presented data on a muscular-dystrophy-associated point mutation of human lamin A that is 'translated' into the single worm lamin (Y59C) and affects muscle-specific promoter release from the nuclear envelope, in a dominant-negative fashion. Failure to release heterochromatin reduces expression from the array-borne myosin promoter, and the effect on endogenous genes compromises muscle integrity. This suggests that a fine-tuned nuclear organization is necessary for proper transcription in somatic tissues in both worms and humans.

To address the regulation of large-scale chromatin organization during interphase, Andrew Belmont's group (U. Illinois, Urbana-Champaign, USA) is using engineered chromosome regions tagged with lac-operator repeats to visualize changes in interphase chromosome structures. By using multiple copy-insertions of bacterial artificial chromosomes (BACs), they can recapitulate transcription and intranuclear positioning of model gene loci and begin to identify *cis*- and *trans*-acting factors to regulate chromosome structure, dynamics and positioning. An RNAi screen in *Drosophila* S2 cells identified potential factors regulating chromatin decompaction following transcriptional activation, and mutation studies in mammalian cell lines identified sequences responsible for targeting loci to nuclear speckles or the nuclear periphery. Such systems will advance our understanding of the factors that regulate large-scale chromatin structure during interphase, modulating transcription regulation.

In addition to the advances in imaging techniques, the chromatin field has benefited immensely from recent developments in high-throughput technologies, which now enable the genome-wide mapping of chromatin modifications, protein association, nucleosome positioning, replication timing, DNase I hypersensitive sites, long- and short-range chromosome interactions, as well as genome–nuclear-lamina interactions.

Analysing such lamina-associated domains (LADs) by using high-throughput technologies, Bas van Steensel (The Netherlands Cancer Institute, Amsterdam)

reported a set of constitutive LADs (cLADs) that are common to several cell types. These were found to be highly conserved in human and mouse, bound by both lamin A and lamin B1, and to have a high AT-content. In collaboration with Harald Herrmann (German Cancer Research Center, Heidelberg), they demonstrated that many of the *in vivo* associations could be recapitulated by *in vitro* binding of the genome to pure lamin-A polymers. This suggests that a global nuclear organization is conserved in evolution and that lamins are at least partly responsible for the binding of cLADs to the nuclear lamina.

Another layer of 3D genome organization that has gained from genomic technologies is the genome-wide mapping of replication timing. Having established that replication timing and 3D-interaction maps (Hi-C) probe a similar level of chromosome organization (Lieberman-Aiden *et al*, 2009; Ryba *et al*, 2010), David Gilbert (Florida State U., USA) reported that large-scale chromatin reorganization is restricted to regions of replication-timing changes. In contrast to the common view, Gilbert's group has shown that developmental changes in replication timing and chromatin reorganization do not accompany changes in chromatin accessibility, and are independent of changes in transcription. Intriguingly, by using such large-scale Replication timing maps, they have identified both pan-specific and patient-specific replication-timing 'fingerprints' for acute lymphocytic leukaemia. This shows that replication timing is a highly regulated process, and that disturbances in it might be predictive of disease.

One mechanism by which disease state or replicative stress can alter replication timing was revealed by Julian Blow (U. Dundee, UK). His group has found that when replication forks are stalled, DNA replication is promoted elsewhere by the activation of otherwise-dormant replication origins within the same replication domain. At the same time, DNA-damage-response kinases activated by the stalled forks preferentially suppress the assembly of new replication factories. This ensures that chromosomal regions experiencing replicative stress complete synthesis before new regions of the genome are replicated.

Cristina Cardoso (Technical U. Darmstadt, Germany) used GFP-tagged replication proteins and ectopically expressed Xist to show the highly synchronous replication of the inactive X-chromosome (Xi)

within 1–2 h during early–mid S-phase in living mammalian cells. Searching for the underlying mechanism, her group found that histone hypoacetylation had a key epigenetic role in controlling synchronous Xi replication. Taken together, these studies not only provide deeper understanding of the known nuclear entities, but also bring forward new concepts in nuclear organization and function.

Mitosis and meiosis

Although interphase comprises the lion's share of the cell cycle, during which chromatin is organized into chromosome territories (Cremer *et al*, 2006), it is during mitosis that chromatin condenses into discrete chromosomal units. David Spector (Cold Spring Harbor, New York, USA) discussed the mechanisms of transcriptional induction on exit from mitosis. Comparing interphase and post-mitotic cells by using live imaging of a single transcribed locus, his group has shown that post-mitotic induction of the same genetic locus occurs more rapidly than interphase induction. Interestingly, transcriptional induction was accompanied by recruitment of RNA polymerase II (RNAPII) large subunit and the bromodomain protein Brd4, which binds to H4K5ac. RNAPII recruitment preceded recruitment of Brd4 before mitosis, but it followed Brd4 recruitment after mitosis, suggesting that H4K5ac might act as a chromatin mark, or 'bookmark', to 'memorize' gene activation. Tethering Brd4 to the interphase locus caused significant chromatin decondensation in the absence of transcription. These data shed new light on the mechanisms that support post-mitotic transcriptional induction and memory.

John Marko (Northwestern U., Evanston, Illinois, USA) presented experiments examining the mechanical properties of mitotic chromosomes. Marko's group has used measurements of the elastic stiffness of metaphase chromosomes as a means by which to study their internal structure. Marko described how experiments with nucleases, proteases and topoisomerases suggest that metaphase chromosomes are effectively 'gels' or 'networks' of chromatin, and chromatin is non-covalently linked by non-histone-protein-based linkages. Such networks might be responsible for regulating chromosome positioning, dynamics and even chromosome pairing during meiosis.

The mechanisms by which chromosomes recognize their homologues during meiosis are not yet understood, but new insights

from the group of Yasushi Hiraoka (Osaka U., Japan) reveal a remarkable role for a meiosis-specific, non-coding, polyadenylated RNA in the pairing process. By monitoring homologous-chromosome pairing in living cells, the group has shown early pairing of a specific chromosome locus during meiotic prophase in *Schizosaccharomyces pombe*. RNA accumulation at the respective gene locus is essential for pairing, and introducing this sequence into other chromosomal sites enhances local pairing at these sites through local RNA expression. These findings demonstrate a role for non-coding RNAs in homologous chromosome pairing and open the door for the identification of additional non-coding RNAs in this fascinating process.

Regulating transcription and epigenesis

Large-scale chromatin and chromosome dynamics have important roles in nuclear function and gene regulation, but it is also clear that local regulation by chromatin-modifying proteins has an essential role in transcriptional activation and repression: through DNA methylation at the genomic level and mostly through histone modifications at the chromatin level.

Heinrich Leonhardt (Ludwig-Maximilians U., Munich, Germany) presented work on the complex role and regulation of DNA methylation during development and disease. It was recently shown that genomic 5-methylcytosine can be further modified to 5-hydroxymethylcytosine (hmC) by a family of developmentally regulated dioxygenases (Tet1, Tet2 and Tet3) in mammalian DNA (Tahiliani *et al*, 2009). The Leonhardt group has developed enzymatic assays to measure hmC levels. Lower hmC levels in tumour cells of patients with secondary acute myeloid leukaemia correlated with *TET2* mutations and altered gene-expression patterns. In some tumour cells, *IDH2* mutations were implicated in the accumulation of 2-hydroxyglutarate (2-HG)—a competitive inhibitor of oxygenases—providing a potentially important link between cancer metabolism and epigenetic gene regulation. The dynamics of hmC and its direct role in disease remain to be examined.

Chromatin-related transcriptional dynamics can be elegantly studied in *Drosophila*. John Lis (Cornell U., Ithaca, New York, USA) presented data to show that poly-ADP ribose (PAR) polymerase (PARP), which is associated with the 5' end of the heat shock factor (*HSF*) gene in *Drosophila*, is redistributed after heat shock, concomitant with the loss

of the nucleosome inside the gene and the accumulation of PAR. His group has also shown that HSF is regulated by an abrupt change in H2A Lys 5 acetylation (H2AK5ac). By knocking down several chromatin-related proteins, they have identified the acetyl transferase—Tip60—and the histone deacetylase—HDAC3—as key regulators of H2AK5 acetylation following heat shock at the *HSF* locus. These findings provide a glimpse into the mechanism that regulates the rapid response to heat shock at specific loci.

No less important than transcriptional induction is transcriptional silencing. Genevieve Almouzni (Institut Curie, Paris, France) discussed recent data showing the targeting of the heterochromatin protein HP1 α to pericentric heterochromatin by SUMOylation. Her group found evidence for long, nuclear non-coding transcripts corresponding to main satellite repeats, of which the forward transcripts are specifically associated with the hinge domain of SUMO-modified HP1 proteins. The hinge domain and its SUMOylation are crucial for the targeting of HP1 α to pericentric domains. These data add to the growing list of the mechanisms of regulation of centromeric chromatin. With respect to silencing, a new model of the PcG bodies in human cells was presented by Jana Šmigová (Charles U., Prague). According to this model, the PcG bodies are not nuclear bodies situated in the inter-chromatin compartment, but correspond to a local accumulation of large-scale heterochromatin fibres, with the heterochromatin fibres being enriched in the PcG-protein immunogold label throughout the nucleus (Šmigová *et al*, 2011).

Evidently, chromatin regulates more than just transcription. Recent, surprising findings have revealed the intriguing link between chromatin and RNA splicing, showing that nucleosomes are preferentially positioned inside exons and that these exons are preferentially marked by H3K36me3 (Schwartz *et al*, 2009; Tilgner *et al*, 2009). Maria Carmo-Fonseca (U. Lisbon, Portugal) has extended these observations to show that genes without introns lack the H3K36 mark, and that splicing promotes H3K36 methylation and Setd2—an H3K36 methyltransferase—recruitment. These data suggest that co-transcriptional splicing is mechanistically coupled to Setd2 recruitment to the elongating RNAPII.

The idea of co-transcriptional splicing has gained further momentum from genome-wide analyses in *Saccharomyces*

cerevisiae by Karla Neugebauer (MPI of Molecular Cell Biology and Genetics, Dresden, Germany). Nascent RNA profiling, aided by computer simulation, has revealed RNAPII pausing within terminal exons of the majority of the intron-containing genes, but not in genes lacking introns or inefficiently spliced genes. These results demonstrate the coupling of transcription and splicing by terminal exon pausing and, on the basis of what we have learnt, call for the testing of whether such terminal exons contain any special nucleosomes that might be responsible for RNAPII pausing.

Coda

Beyond the outstanding presentations, all participants enjoyed warm hospitality, a friendly atmosphere and nightly beer and wine sessions, which stimulated scientific discussions and the exchange of ideas. During the musical enlightenment on the last evening—provided by the Martinu quartet performing Dvorak's string quartet in G major, Op. 106—people tuned in to the conviction that what was once regarded as a static scaffold for gene expression is now emerging as a highly dynamic, regulated and complex machinery that is involved in every aspect of the life cycle of the cell, as well as in the functional coordination of cells within tissues.

ACKNOWLEDGEMENTS

We apologize to all participants whose work was not included due to space limitations. The authors are supported by the Edmond J. Safra Foundation (E.M.), the German-Israeli-Foundation (GIF) grant 111/2007 (H.H.) and grant number MSM0021620806 (I.R.).

REFERENCES

- Cremer T *et al* (2006) *Curr Opin Cell Biol* **18**: 307–316
- Lieberman-Aiden E *et al* (2009) *Science* **326**: 289–293
- Ryba T *et al* (2010) *Genome Res* **20**: 761–770
- Schwartz S, Meshorer E, Ast G (2009) *Nat Struct Mol Biol* **16**: 990–995
- Šmigová J *et al* (2011) *Nucleus* **2**: 219–228
- Tahiliani M *et al* (2009) *Science* **324**: 930–935
- Tilgner H *et al* (2009) *Nat Struct Mol Biol* **16**: 996–1001
- Wolffe AP, Hansen JC (2001) *Cell* **104**: 631–634

Eran Meshorer is at The Hebrew University of Jerusalem, Israel. Harald Herrmann is at the German Cancer Research Center (DKFZ), Heidelberg, Germany. Ivan Raška is at the Charles University, Prague, Czech Republic. E-mails: meshorer@cc.huji.ac.il; h.herrmann@dkfz-heidelberg.de

Published online 15 July 2011

EMBO reports (2011) **12**, 748–750.
doi:10.1038/embor.2011.148