Meeting report: mitosis and nuclear structure

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The Company of Biologists Workshop entitled 'Mitosis and Nuclear Structure' was held at Wiston House, West Sussex in June 2013. It provided a unique and timely opportunity for leading experts from different fields to discuss not only their own work but also its broader context. Here we present the proceedings of this meeting and several major themes that emerged from the crosstalk between the two, as it turns out, not so disparate fields of mitosis and nuclear structure. Co-chaired by Katherine Wilson (Johns Hopkins School of Medicine, Baltimore, MD), Timothy Mitchison (Harvard University, Cambridge, MA) and Michael Rout (Rockefeller University, New York, NY), this workshop brought together a small group of scientists from a range of disciplines to discuss recent advances and connections between the areas of mitosis and nuclear structure research. Several early-career researchers (students, postdoctoral researchers, junior faculty) participated along with 20 senior scientists, including the venerable and affable Nobel Laureate Tim Hunt. Participants were encouraged to embrace unconventional thinking in the 'scientific sandbox' created by this unusual combination of researchers in the inspiring, isolated setting of the 16th-century Wiston House.

The tone of the meeting was set by Katherine Wilson during the initial session in which she emphasized that many cellular elements have been functionally, and perhaps inappropriately, pigeonholed. For example, it has long been thought that the nuclear envelope had to be broken down and kept away from the mitotic spindle during mitosis in higher eukaryotes, but there is now a compelling and growing body of evidence that nucleoskeletal components play active roles in mitosis. She stressed that these emerging links raise questions about the evolution of both nuclear structure, and genome partitioning. This meeting was therefore specifically designed to consider the overlap between the subject areas of evolution, the nucleoskeleton and nuclear pore complexes (NPCs), and mitosis (Fig. 1).

What evolution and bacteria can tell us about the origins of nuclear structure

Prokaryotes, which have no nucleus, highlighted the holistic and evolutionary approach of this conference. David Sherratt (University of Oxford, UK) presented his group's fascinating work on how bacteria segregate their DNA, particularly focusing on the relationship between DNA replication and repair. First, he described an intriguing RecA-mediated DNA repair mechanism that bundles genomic loci, including loci in sister cells, to act as a matrix for homology search. Then he explained work on the SMC complex MukBEF and its dynamic role in origin positioning.

The question of why cells actually need a nucleus came up during the discussion following a talk by Christine Jacobs-Wagner (Yale University, New Haven, CT) describing chromosome segregation in the bacteria *Caulobacter crescentus*. This bacterium uses a partitioning (Par) apparatus to accurately divide its genetic material. Through a combination of biochemistry and advanced imaging techniques, her group has been able to show that DNA segregation occurs through a multipoint interaction between ParA and ParB to allow the directed transport of a newly replicated origin of replication away from its copy and towards the opposite end of the cell. Hence, multivalent interactions, a key feature of the eukaryotic chromosome segregation mechanism, appear to be a recurring theme of directional DNA transport.

Many participants received their first introduction to *Gemmata obscuriglobus*, a compartmentalized soil bacterium, in a talk by Damien Devos (Ruprecht-Karls University, Heidelberg, Germany). In *Gemmata*, a double membrane, highly reminiscent of a nuclear envelope, appears to surround the bacterial DNA. However, through careful 3D reconstructions (EM tomography), the Devos group discovered the apparently separate compartments are, in fact, interconnected. Thus, although this bacterium lacks a nucleus, this marks the first description of a tubulovesicular network in bacteria that mediates endocytosis-like protein uptake. He hypothesized that the Planctomycetes-Verrucomicrobia-Chlamydiae bacterial superphylum could be the precursor of eukaryotic cell organization.

Nuclear intermediate filaments (lamins) are the major structural element associated with the nuclear envelope (NE) in animal cells, and their apparent absence in divergent eukaryotes is perplexing. Two speakers provided evidence for this missing 'piece' of the evolutionary puzzle. Ralph Gräf (University of Potsdam, Germany) spoke about a new centrosome-binding protein in *Dictyostelium*, named NE81. NE81 contains a predicted central α-helical rod domain, similar to intermediate filament proteins, and other conserved features, indicating an evolutionary relationship to lamins. NE81 localizes at the NE throughout the cell cycle. During interphase, this protein is largely immobile and thus assembled, and is disassembled by phosphorylation of a putative cyclin-dependent kinase 1 (CDK1) site during closed mitosis. The disassembled form continues to associate with the NE, possibly because of permanent prenylation of the C-terminal CaaX box. Furthermore, knockout of the NE81 gene affected chromatin condensation and centrosome attachment to the nucleus in *Dictyostelium*. There was general agreement that NE81 is the *Dictyostelium* lamin. Mark Field (University of Cambridge, UK) described a lamin-like protein as well as nuclear pore complex proteins in another single-celled eukaryote, *Trypanosoma brucei*. The protein NUP-1 has a long α-helical rod domain and localizes at the nuclear envelope. Knockdown of NUP-1

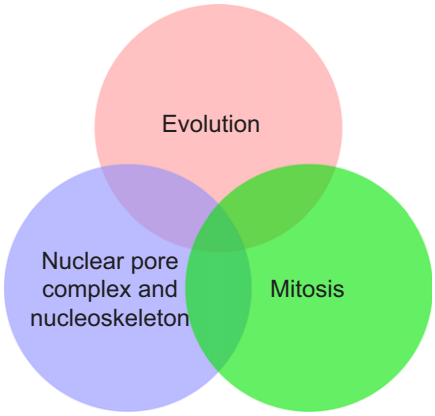

Fig. 1. A Venn diagram showing the three main fields represented at the Workshop and their overlap.

expression disrupts nuclear shape and disorganizes NPCs and chromosomes. NUP-1 is also required for gene silencing at the nuclear periphery, especially genes encoding variant surface glycoproteins (VSGs) that are located near telomeres. These studies of NE81 and NUP-1 suggest that divergent eukaryotes have proteins that are structurally and functionally similar to lamins and might provide further insight into other conserved nucleoskeletal components.

In a related talk, David Evans (Oxford Brookes University, UK) highlighted the current state of knowledge regarding the nuclear structure of higher plants. He summarized recent findings regarding SUN and KASH protein complexes – the physical linkages that bridge the nucleoskeleton and cytoskeleton (Zhou et al., 2012). He went on to discuss the make-up of the nucleoskeleton in plants, which interestingly lacks known homologs of lamins. This was followed by presentation of data supporting the claim that members of the LINC (little nuclei) protein family, LINC1 and LINC2, might function as components of the missing nuclear lamina in plant cells.

The decidedly non-nuclear functions of nuclear proteins

The diverse functions of nuclear lamins

A recurring theme during this meeting was that many proteins historically defined as either ‘mitotic’ or ‘nuclear’ can, and do, function in both contexts (as well as others). Several talks described surprising and decidedly non-nuclear functions of lamins, NPC proteins and NE proteins (e.g. SUN–KASH complexes). Yosef Gruenbaum (The Hebrew University of Jerusalem, Israel) spoke about lamins in the nematode *Caenorhabditis elegans*. Lamins are particularly important for human health, because mutations in lamins cause many human diseases (‘laminopathies’). *C. elegans* mutants lacking either LEM2 or emerin (nuclear inner membrane proteins that bind lamin) had elongated transition zones in the gonad, due to defects in DNA repair. Thus, nuclear ‘lamina’ proteins are required for DNA repair.

Yixian Zheng (Carnegie Institution for Science, Baltimore, MD) spoke about her laboratory’s discovery that lamins are dispensable for self-renewal and pluripotency of mouse embryonic stem cells, but have fundamental roles in organogenesis. A double knockout of both B-type lamin genes (*Lmnb1* and *Lmnb2*) in mouse resulted in defective spindle orientation in neural stem cells in embryos, which might explain, in part, why these mice have defects in brain development. B-type lamins are also required to form the *Drosophila* testis, and function specifically in cyst stem cells to support male germline development. Together, these findings support the idea that B-type lamins have tissue-specific functions during development.

Kristen Johansen (Iowa State University, Ames, IA) spoke about the spindle matrix, a collection of proteins that, in addition to microtubules, regulate spindle morphology and function. Her talk focused specifically on the roles of EAST and Megator, which bind to the NE before mitosis, yet during spindle assembly, form an elastic, gel-like matrix. Importantly, depletion of either component negatively affects the fidelity of chromosome segregation. Consistent with the idea of proteins being repurposed from interphase to mitosis, Megator, a protein that associates with the NPC and spindle matrix, is required for the proper localization of the spindle assembly checkpoint component Mad2.

The diverse functions of NPC proteins

Co-organizer Michael Rout gave a thorough overview of NPC structure, including the use of crosslinking and mass spectrometry of yeast NPC proteins to resolve NPCs at close to atomic resolution *in vivo* (Fernandez-Martinez et al., 2012). NPCs were previously thought to function solely as conduits for molecular transport between the nucleoplasm and cytoplasm. Like lamins, however, several talks featured NPC proteins in diverse new roles including active participation in mitosis.

Valérie Doye (Institut Jacques Monod, CNRS and University of Paris Diderot, France) discussed the non-canonical roles of the Nup107-160 complex (comprising seven subunits; also called the ‘Y-complex’), a key player in NPC assembly. In mitosis, this complex localizes at kinetochores and is important for chromosome alignment and segregation. One component of the Y-complex, namely Nup133, is further required for dynein recruitment to the NE and centrosome positioning in prophase. Nup133 also contributes to mouse ES cell differentiation although the underlying mechanisms are still unknown. Finally, the Y-complex also dynamically localizes in nuclear bodies in some interphase cells, suggesting additional non-canonical intra-nuclear function(s).

Catherine Dargemont from the same institute described how NPC post-translational modifications, particularly ubiquitylation, are important for cell cycle progression in yeast (Hayakawa et al., 2012). Although the reason is still unclear, it is interesting that nucleoporins are prevalently monoubiquitylated by different components of the ubiquitin-conjugation machinery. She introduced the concept of nucleoporin ubiquitylation as a timing machine that modulates the diverse functions of the NPC, and in particular mRNA export pathway from the nucleus.

Expanding the list of alternative NPC functions even further, Erica Golemis (The Fox Chase Cancer Center, Philadelphia, PA) ‘brought together’ nucleoporins, mitosis and the primary cilium in cancer. All human cells carry a small sensory cilium that mediates

signaling from the extracellular environment. NPC proteins are located at the base of the cilium, and regulate molecular traffic into and out of the cilium. Before cells enter mitosis, the cilium is disassembled so that its basal body can move toward the nucleus and function as the centrosome. She presented data using a ciliary re-absorption system in RPE cells. This work showed that at the G2/M boundary, the NEDD9 protein is required for Aurora A activation directly at the basal body. She also emphasized growing links between defects in nucleoporin function and cancer.

The diverse functions of nuclear membrane proteins

Several speakers discussed SUN- and KASH-domain-containing proteins. Brian Burke (Institute of Medical Biology, Singapore) described the interplay between chromosomes and nuclear membrane proteins in animal cells. His presentation was focused on the role of LINC (links the nucleoskeleton and cytoskeleton) complexes, which, as noted above in plants, are composed of SUN-domain proteins and KASH-domain proteins (aka nesprins). Nesprins can bind the actin cytoskeleton and/or specific microtubule-based motors, namely kinesin-1 and cytoplasmic dynein. In this way, motor proteins can move LINC complexes, often resulting in a polarized distribution within the nuclear envelope. Brian Burke went on to present data describing how specific LINC complexes associate with chromosomes, particularly the SUN1–KASH5 complex. Here, SUN1 is required to mediate LINC tethering to telomeres inside the nucleus, whereas KASH5 recruits and binds to dynein in the cytoplasm. In this way, he proposed, the SUN1–KASH5 complex is able to facilitate dynein-dependent chromosome-localization during mitosis by an outside-in mechanism.

Crosstalk between nuclear proteins and gene expression was discussed by Eric Schirmer (University of Edinburgh, UK). His group has shown that over half of all nuclear envelope transmembrane proteins (NETs) are tissue restricted (Korfali et al., 2012) and the evolution of this class of proteins correlates with organismal diversity and complexity. The additional observation that altering the expression of tissue-specific NETs causes chromosome re-organization (Zuleger et al., 2013) strongly argues for the hypothesis that NETs represent a driving mechanism in establishing tissue-specific patterns of spatial genome organization during differentiation. However, the molecular details behind this are still unknown. Although it appears that a mitotic cycle is needed for NETs to facilitate this re-organization, not much is known about the localization or interaction of this class of proteins during mitosis. This aspect will clearly represent a challenging avenue for the future.

How the morphology of the NE affects the accuracy of chromosome segregation was discussed by Martin Hetzer (Salk Institute for Biological Sciences, San Diego, CA). He presented recent evidence implicating micronuclei in the generation of genome instability (Hatch et al., 2013). Micronuclei underlie a major genome instability phenomenon, termed chimotrypsis, in cancer cells (Crasta et al., 2012). Micronuclei also have defective nuclear membranes and defects in both DNA damage repair and replication. The advance provided by Martin Hetzer's group is to clearly show that the abnormal nuclear structure present in the micronuclei is the main reason for this instability. During interphase, micronuclei experience sudden NE rupture, which (unlike the main nucleus) is not repaired, probably due to a micronucleus-specific defect in laminB1. LaminB2 restores normal function and reverts epigenetic changes associated with this abnormal behavior. It will be exciting in the future to understand how this NE defect in the micronuclei originates. This new direction already represents a very important finding because disrupted micronuclei could provide a new biomarker for the genome instability associated with cancer progression.

The diverse functions of 'mitotic' proteins (and chromosomes)

Thus far, this report has focused on nuclear proteins moonlighting as surprising effectors of seemingly unrelated, non-nuclear pathways. However, this workshop had another theme: the idea that proteins and cellular structures long considered mitotic can have other unexpected roles. Tim Mitchison (Harvard University, Cambridge, MA) described a refinement of the *Xenopus* egg extract system that combines these extracts with quantitative mass spectroscopy to characterize selected groups of mitotic proteins. This new approach allowed them to analyze perturbation-dependent changes in the protein composition of large microtubule structures – called 'pineapples' because of their shape (Mitchison et al., 2013) – that share many characteristics of mitotic spindles. By comparing the proteomes of pineapples assembled under different conditions, this approach can be used to classify proteins with both mitotic and nuclear functions that would not be readily found by classical genetic approaches.

Rebecca Heald (University of California, Berkeley, CA) spoke about cell-cycle-dependent changes in endoplasmic reticulum (ER) and chromosome interactions. In addition to its role in segregating chromosomes, she hypothesized that the mitotic spindle has a mechanism that ensures organelles are not trapped inside the re-forming nucleus. Using a biochemical screen, she identified receptor expression-enhancing protein 4 (REEP4), which associates with both membranes and microtubules (Schlaitz et al., 2013). RNAi-mediated knockdown of REEP4 and the closely related protein REEP3 caused the premature localization of ER on mitotic chromosomes at metaphase, resulting in daughter cells that contain intranuclear membrane structures. These results suggest REEP3/4 proteins are required to clear ER away from metaphase chromosomes during mitosis.

Mary Dasso (NIH, NICHD, Bethesda, MD, USA) discussed the regulation of Ran during mitosis. Ran is a small GTPase that controls the directionality of interphase nucleocytoplasmic transport, as well as mitotic spindle assembly. RCC1, the guanine nucleotide exchange factor for Ran ('recharges' Ran to the GTP-bound state), binds chromatin but also localizes at centromeres. She showed a 'wave' of RCC1 associating with chromatin at the metaphase to anaphase transition and demonstrated that the association of RCC1 with chromatin is modulated through the formation of a heterotrimeric complex between RCC1, Ran-GTP and a Ran-GTP binding protein, RanBP1. She also discussed results indicating that the phosphorylation of RanBP1 drives changes in RCC1 dynamics during anaphase.

Keeping with the theme of non-canonical functions of chromosomes, Tom Misteli (NIH, NCI, Bethesda, MD) discussed the role of chromatin structure and DNA repair. He proposed a novel link between chromatin conformation and DNA repair signal transduction. The DNA damage response involves activation of the ataxia telangectasia-mutated (ATM) kinase and phosphorylation of several targets including the histone H2A variant γH2AX (Burma et al., 2001). Although chromatin compaction moderately affects access and repair of double-strand DNA breaks, it strongly stimulates the DNA break signaling (Murga et al., 2007). For example, mitotic cells treated with agents inducing double-strand DNA breaks activate a primary DNA damage response that comprises early signaling events, including activation of ATM and a second kinase DNA-PK, and histone H2AX phosphorylation but no detectable recruitment of the E3 ubiquitin ligases RNF8 and RNF168, or accumulation of 53BP1 and BRCA1, at double-strand DNA break sites (Giunta et al., 2010). His new hypothesis is that a specialized condensed chromatin conformation is sufficient to trigger early signaling of the repair machinery even in the absence of damage. This form of chromatin is sufficient to activate ATM but there is no recruitment of downstream factors.

Concluding remarks

The idea that evolution is resourceful and has repurposed cellular components to serve multiple functions is certainly not new; however, this workshop highlighted that historical classifications around mitosis and nuclear structure need to be reconsidered in light of this. Therefore, the challenge to both established and new investigators alike is to reassess their research with a broader perspective and a heightened awareness of potential links between traditionally segregated subject areas. The workshop also revealed that despite our current level of understanding, much remains unknown. For example, the structures of many nucleus-associated protein assemblies have been elucidated with impressive resolution; however, a large gap still remains in understanding the basis for their diverse functions. Furthermore, the mechanistic links between nuclear proteins and the plethora of conditions and diseases that result from their perturbation remains poorly understood. These gaps in our knowledge represent intriguing frontiers and it is particularly exciting to think about what will emerge from research at the confluence of seemingly disparate fields.

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