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MEETING REPORT

Meeting report – Mitotic spindle: from living and synthetic systems to theory

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ABSTRACT

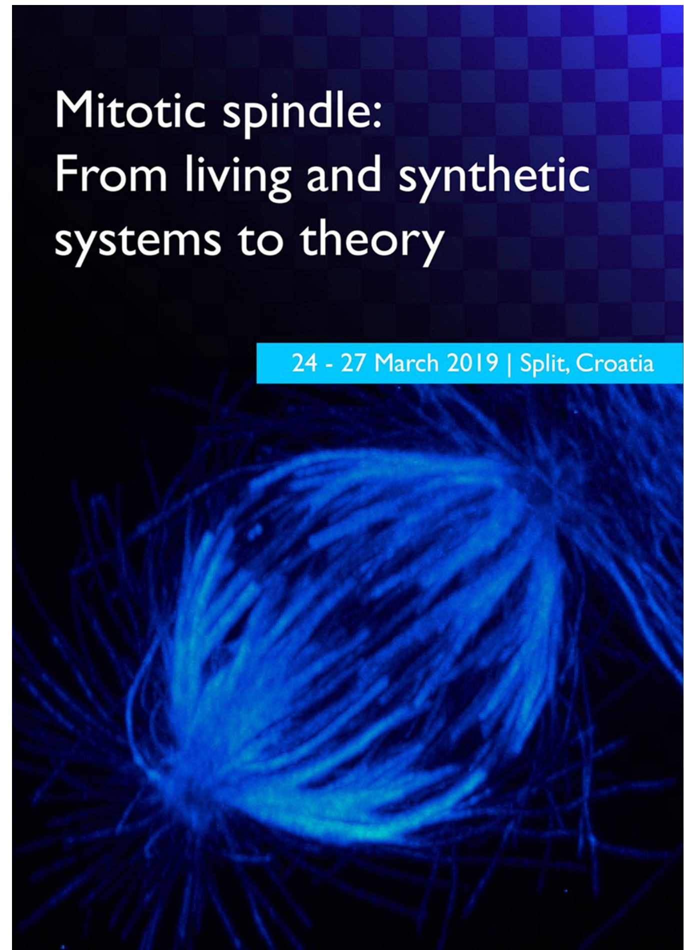
Leading scientists from the field of mitotic spindle research gathered from 24–27 March 2019 to participate in the first 'Mitotic spindle: From living and synthetic systems to theory' conference. This meeting was held in Split, Croatia, organized by Nenad Pavin (Faculty of Science, University of Zagreb) and Iva Tolić (Ruđer Bošković Institute, Zagreb). Around 75 participants presented the latest advances in mitotic spindle research, ranging from live-cell imaging, *in vitro* reconstitution experiments and theoretical models of spindle assembly. The meeting successfully created an environment for interesting scientific discussions, initiation of new collaborations and development of fresh ideas. In this report, we will highlight and summarize new data challenging the established models of spindle architecture, advances in spindle reconstitution assays, discovery of new regulators of spindle size and shape as well as theoretical approaches for investigating motor protein function.

Introduction

Even though the mitotic spindle has been the subject of research efforts for more than a century, fundamental questions regarding its architecture and function remain unanswered. Recent advances in microscopy, molecular biology and theoretical approaches that provide new insight into some of these questions were the motivation behind the organization of the first 'Mitotic spindle: from living and synthetic systems to theory' conference in Split, Croatia. The goal of the meeting, divided into ten sessions over four days, was to bring leading scientists, PhD students and postdocs studying the spindle together, present their new findings and exchange ideas with colleagues. The relatively small number of participants allowed for a relaxed atmosphere with sufficient time for in-depth discussions on the different subjects covered at the meeting. These include spindle architecture and mechanics, kinetochore structure, evolution and function, spindle assembly, morphology and positioning, mechanisms of microtubule flux, stability and nucleation, the role of kinesins in mitosis, *in vitro* approaches to investigating the spindle, physical models for spindle structure and chromosome positioning and new tools and methods based on gene editing and directed evolution for examining the mitotic spindle.

Spindle architecture and mechanics

Richard McIntosh (University of Colorado, Boulder, USA) kick-started the meeting on a long-standing question: what do the ends of dynamic microtubules (MTs) look like in the spindle? Using electron tomography to examine growing MTs in anaphase B spindle midzones from six different species, they found that plus-ends of growing MTs exhibit curved protofilaments that are similar in appearance to depolymerizing MTs (McIntosh et al., 2018). None of the MTs had the sheet-like ends that were previously associated with growing MTs. These curved protofilaments vary in shape, suggesting that they exhibit Brownian movement; in addition, they could provide pulling or pushing forces on kinetochores through associated non-motor microtubule-associated proteins (MAPs). Continuing with electron microscopy, Thomas Müller-Reichert (TU Dresden, Germany) showed tomography images of metaphase spindles of the first mitotic division in the *C. elegans* embryo (Redemann et al., 2017). In these spindles, more than 8000 MTs emanate from each centrosome, and less than 10% are kinetochore



Conference poster. This displays a metaphase spindle from a HeLa cell stained with tubulin antibody and imaged using expansion microscopy. ©Tolić lab.

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Welcome drinks at the terrace of the Radisson Blue hotel, with the Adriatic Sea and Šolta island in the background.

MTs (kMTs). MTs are randomly connected to the holocentric chromosomes but their number correlates with chromosome size. Unexpectedly, not all kMTs have their minus-end at the spindle pole. Instead, some are anchored in the MT network. He next presented ongoing work on the HeLa cell metaphase spindle, where 15% of all MTs are kMTs. They contain seven to eight, and not 15–20, MTs per k-fibre, as described previously (Wendell et al., 1993). Similar to the *C. elegans* spindle, not all k-fibres had MT minus-ends located at the poles. In light of this data, the 'stiff-rod' model of k-fibres – with MTs continuously spanning from the kinetochore to the spindle pole – is probably incorrect.

How does the mammalian spindle with all its dynamic parts maintain a steady-state, robust structure? Sophie Dumont (University of California, San Francisco, USA) first showed that the clustering of MT minus-ends by nuclear mitotic apparatus protein (NuMa) and the motor protein dynein is essential for the spindle to reach a steady-state structure (Hueschen et al., 2019). Without either of these proteins, the spindle is turbulent. Second, using microneedle manipulation, she showed that the spindle reinforces its structure in its centre, where it is most dynamic, and locally dissipates force at its dynamic interfaces, suggesting two strategies for preserving global spindle structure. Marin Barišić (DCRC, University of Copenhagen, Denmark) examined the contribution of motors and MAPs to poleward flux using photoactivation methods. Deletion of the chromokinesin Kif4A reduces the flux in late prometaphase and metaphase. In contrast, Ndc80 depletion increases flux, indicating that stable kinetochore–MT end-on attachments work as flux brakes. Depletion of NuMA and HSET (also known as KIFC1), but not depletion of the antiparallel MT crosslinker protein regulator of cytokinesis 1 (PRC1) reduced MT-flux rates only when Ndc80 was present and end-on attachments were established. Thus, MT-crosslinking molecules ensure the equal distribution of MT-flux-dependent forces across the mitotic spindle. Sebastian Furthauer (Flatiron Institute, New York, USA) described work with a reconstituted system of MTs, carboxy-terminal kinesin 2 (XCTK2) motors and *Xenopus* meiotic spindles to address how large-scale behaviours of actively crosslinked networks emerge from the properties of their constituents (Furthauer et al., 2018 preprint). Photobleaching experiments in both systems demonstrated that MTs that are aligned by motors continually slide past each other at a speed independent of the local MT polarity. This is consistent with a theoretical model where MTs are a heavily crosslinked gel in a dense regime with their length being significantly greater than the spacing between them. Mihaela Jagrić (Ruđer Bošković Institute, Zagreb, Croatia) presented new data on the role of PRC1 in metaphase. Using an optogenetics approach (Milas et al., 2018), she examined the structural changes in the spindle upon removing PRC1 in metaphase. Her results show that PRC1 removal, (i) partially disassembles the bridging fibres, MT bundles that connect sister k-fibres, (ii) results in a less-rounded spindle, (iii) decreases the inter-kinetochore distance, and (iv) widens the metaphase plate. During anaphase, cells with sequestered PRC1 exhibit a higher percentage of lagging kinetochores. Jagrić highlighted that, despite the similarities of the phenotypes between what was found for PRC1 RNAi and optogenetics experiments, metaphase plate widening is visible only with optogenetics treatment, suggesting that compensatory mechanisms may mask this phenotype during long-term RNAi treatment.

Jing Xie (Institute Jacques Monod, Paris, France) elucidated the physical forces that maintain spindles in the centre of large cells, where spindles do not contact the cortex. She probed spindles in sea urchin embryos *in vivo* using magnetic tweezers. By applying forces parallel or perpendicular to the spindle axis, she found that bulk cytoplasmic viscoelastic forces maintain the spindle at the cell centre. Thus, the mitotic spindle can be stabilized in the middle of a large animal cell by a MT-contact-independent mechanism. Dan Needleman (Harvard University, Cambridge, USA) used biophysics and quantitative genetics to test models that describe how the spindle acquires its final position and size, such as the timer model, boundary model or limited compound model. Rejecting several previous models, his research group arrived at a new theory of how spindle elongation and positioning – and their scaling with cell size – arise from known biochemical properties of MTs and motors. This resulted in a quantitative understanding of how cortical pulling forces can stably position the spindle, revealed the genetic basis of variations in spindle behaviours among wild *C. elegans* isolates, and provided a mechanistic explanation for the evolution of the spindle between different species of nematodes. Jan Brugués (MPI of Molecular Cell Biology and Genetics, Germany) discussed how forces are propagated in spindles by studying the *Xenopus* meiotic spindle. The spindle consists of two overlapping populations of MTs moving with respect to each other towards the spindle poles. Is the movement due to local MT sorting or long-range force propagation? By combining laser ablation with fluorescence-speckle microscopy, Brugués showed that long-range force transmission is necessary for MT poleward movement. Simulations reveal that when there is <1 motor per MT, no coherent flows are observed. Above a critical threshold of approximately four motors per MT, the structure gelates, and coherent flows that span the whole structure emerge.

It was clear from the very first talk of the meeting that previous models of spindle architecture, from the 'stiff-rod' model of kMTs to mechanisms underlying positioning and stability of the spindle, are undergoing major revision as the spindle continues to be probed with new tools in a range of model systems.

Building a spindle *in vitro* from its parts

Several groups discussed their efforts at reconstituting the subreactions underlying spindle organization and function. Thomas Surrey (Center for Genomic Regulation, Barcelona, Spain) and Radhika Subramanian (Harvard Medical School, Boston, USA) discussed mechanisms that regulate the organization of the antiparallel MT arrays that form the anaphase spindle. Surrey described the reconstitution of stable antiparallel bundles from three components: soluble tubulin, PRC1

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and the kinesin-4 protein Kif4a, which suppresses MT dynamics (Hannabuss et al., 2019). The length of the midzone is controlled by the PRC1 to Kif4A ratio. Experiments and simulations suggest that the mechanism underlying the organization of this structure involves the synergy between several activities, such as antiparallel MT crosslinking, suppression of MT dynamics and relative MT sliding. Subramanian closely examined MT sliding mediated by the PRC1–Kif4A complex (Wijeratne and Subramanian, 2018). They observed that sliding stalls when MT plus-ends arrive at close proximity due to steric hindrance from proteins accumulating at these MT ends, thus resulting in the formation of a stable crosslinked antiparallel bundle. These experiments unexpectedly reveal how the micron-scale geometrical features of MT arrays such as initial overlap lengths regulate both the dynamic properties of the system, such as relative sliding velocity and the final architecture of a MT bundle.

Junichiro Yajima (University of Tokyo, Tokyo, Japan) discussed another key cytokinesis factor, centralspindlin, a complex of mitotic kinesin-like protein 1 (Mklp1) and the GTPase-activating protein (GAP) Cyk4. High-speed atomic force microscopy (AFM) studies suggested that Cyk4 regulates Mklp1 activity (Davies et al., 2015). To determine the underlying mechanism, they suspended MT away from the coverslip and tracked movement of bead-labelled Mklp1. In the absence of Cyk4, persistent left-handed helical movement is observed. The addition of Cyk4 leads to both left- and right-handed movement. Yajima suggested that Cyk4 may allow for more flexible choice of stepping direction and enable centralspindlin clusters to move effectively through crowded regions of the anaphase spindle. On the theme of *in vitro* studies of mitotic motors, Thibault Legal (University of Edinburgh, Edinburgh, UK) presented structural insights into centromere-associated protein E (CENP-E) (a kinesin-7) targeting to kinetochores. He determined that this 230 nm long plus-end directed kinesin is a V-shaped molecule using small-angle X-ray scattering (SAXS) and rotary shadow electron microscopy (EM). Legal identified the minimal kinetochore-targeting domain of CENP-E and showed that it is sufficient to bind to the pseudokinase domain of BubR1. Whereas the domain of CENP-E that interacts with BubR1 is monomeric, the CENP-E kinetochore-targeting domain requires dimerization to form the BubR1-binding site.

James Wakefield (University of Exeter, Exeter, UK) and Simone Reber (Humboldt-Universität, Berlin, Germany) focused on the α, β-tubulin herterodimer, the basic building block of MTs and the spindle. Reber's research group asks a fundamental question – how does the biochemistry of the α,β-tubulin heterodimer contribute to spindle morphology? Comparative analysis of *Xenopus laevis* and *tropicalis* provides a method for examining mechanisms underlying spindle scaling. Examining MT dynamics *in vitro* using *X. laevis* and *X. tropicalis* tubulin showed that *X. laevis* tubulin grows faster and barely exhibits any catastrophe events, whereas *X. tropicalis* tubulin has a slower growth rate and increased catastrophe rates. Tubulin from these two sources have no qualitative differences in isoforms or post-translational modifications (PTMs) except for phosphoserine, which seems to be present only in the *X. laevis* tubulin. Wakefield presented new data having purified the MT-nucleating complexes augmin and γ-tubulin ring complexes (γTuRC) from *Drosophila* embryos using a novel affinity purification method based on a cleavable protein tag. Bulk polymerization assays showed that augmin has no effect on MT polymerisation alone but enhances MT nucleation mediated by γTuRC. They imaged the outcome of the above reactions and observed MT bundles and branches with rather disorganized architecture in the presence of augmin and γTuRC. To image the MT branches, they combined augmin-bound green fluorescent MTs and γTuRC-associated Rhodamine-labelled MTs. Remarkably, they saw red MTs linked to green MTs, which is reminiscent of the product of a branched templated MT. Their next steps are the localization of the proteins at the ultrastructural level and real-time imaging of branched MT nucleation *in vitro*.

Although reconstitution-based approaches are still far away from being able to reconstruct the spindle from purified proteins, a lot of progress has been made towards elucidating how motors, MAPs and the diversity in tubulin contribute to microtubule organization in the spindle. As more complex reconstitutions are undertaken in the future, a major consideration to take into account is mitosis-specific post-translational modifications of these proteins and how they alter their activity.

Kinetochores – components, structure and evolution

How do cellular traits diversify? To address this question, Geert Kops (Hubrecht Institute, Utrecht, Netherlands) studies kinetochore evolution by (i) mapping kinetochore diversity in eukaryotes using comparative genomics, (ii) tracing kinetochore evolution and (iii) screening for novel functional features (van Hooff et al., 2019). After pointing out that most of our knowledge comes from a very few representatives of the tree of life, he showed examples of organisms with strikingly divergent kinetochore structures. The flagellated protozoan kinetoplastids, for example, have completely rewired their kinetochores during evolution. The excavate *Carpediemonas membranifera* have virtually all known kinetochore-like proteins, whereas the alveolates *Tetrahymena thermophile* and *Perkinsus marinus* have only the chromatin- (CENP-A) and MT-binding (Ndc80/Nuf2-like) proteins, but nothing else. Comparisons of kinetochore constituents from different taxonomic groups indicated that the last eukaryotic common ancestor already had a kinetochore similar to the one we see in humans today. The origin of kinetochore proteins is diverse and they come from different cellular pathways and compartments such as MT-organizing centres (MTOCs), and nucleotide sensing, DNA repair and/or replication, vesicle trafficking, transcription and splicing pathways. This nicely exemplifies how these proteins were co-opted to build the mammalian kinetochore. Nigel Burroughs (Warwick University, Coventry, UK) presented work on determining the architecture of the human kinetochore through measuring 3D distances between proteins using light microscopy. Interpretation of these data requires handling of two key problems: the chromatic shift and inflation of 3D distances (overestimation is high for small distances). They extracted corrected Euclidean distances, achieving localization accuracies of 2–4 nm depending on the fluorophores. Burroughs highlighted that their next challenge is to link these measurements to structural data. Daniela Cimini (Virginia Tech, Blacksburg, USA) discussed new results on the contribution of centromere stiffness on chromosome and MT dynamics. They found that in washout experiments with the Eg5 kinesin motor inhibitor S-trityl-L-cysteine (STLC), cells that have been treated with the histone deacetylase inhibitor trichostatin A (TSA), which reduces chromosome condensation and increases elasticity, take twice as long to establish spindle bi-orientation. Predictions from a mathematical model indicated that an increase in centromere stiffness would suppress chromosome oscillations at the metaphase plate and vice versa. Their preliminary tracking of kinetochores supports these predictions. These results indicate that the centromere can generate forces that, through the kinetochore, can affect MT and spindle dynamics. Jason Stumpff (University of Vermont, Burlington, USA) discussed chromosome alignment and

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the role of Kif18A, which localizes to the plus-ends of kMTs, promotes kinetochore–MT attachments and is required to satisfy the spindle assembly checkpoint in several cell types. What is the mechanistic basis for this? They hypothesized that Kif18A increases kinetochore tension, or that Kif18A recruits protein phosphatase 1 (PP1) and this promotes kMT attachment. Experiments with mutations in the PP1-binding region of Kif18A suggest that PP1 recruitment contributes to checkpoint satisfaction.

Ekaterina Grishchuk (University of Pennsylvania, Philadelphia, USA) provided a critical evaluation of theoretical models for the correction of erroneous MT–kinetochore attachments by Aurora B kinase. The most successful model relies on the bi-stability of the kinase–phosphatase system, characterized by an abrupt transition between stable states of low or high kinase activity. Bi-stability can explain the steep kinase activity gradient observed at the outer kinetochore under pulling forces from the end-on attached MTs (Trivedi et al., 2019). The model predicts that Aurora B bound to MTs spanning the inter-kinetochore centromeric region, such as in prometaphase or merotelic attachments, gets activated by chromatin-bound Aurora B. This activated kinase then diffuses along merotelic MTs and phosphorylates kinetochore substrates, thereby destabilizing the erroneous MT–kinetochore attachments. Vladimir Volkov (from the research group of Marileen Dogterom, Delft University of Technology, Delft, Netherlands) showed elegant bottom-up reconstitutions of the spindle–MT interface, which was performed in collaboration with the group of Dogterom and Pim Huis In 't Veld from Andrea Musacchio's laboratory (MPI of Molecular Physiology, Dortmund, Germany). They showed that oligomers of the Ndc80 complex rescue MT shortening, thereby reconstituting the 'gripping' function of kinetochores. Phosphorylation of the Ndc80 N-terminal tail by Aurora B reduces the duration of MT stalling and prevents rescue. Binding of the spindle- and kinetochore-associated (Ska) complex to Ndc80 can overcome the destabilizing action of Aurora B by increasing the duration of Ndc80-mediated MT stalls. Thus, the balance between MT rescue and detachment at the kinetochore is regulated by phosphorylation and by Ska–Ndc80 interactions (Huis in't Veld et al., 2019 preprint). Taken together, there were updates to multiple aspects of the kinetochore at the meeting, from the list of its parts to biochemical mechanisms to cellular structure, dynamics and evolution of kinetochores.

Centrosome and spindle assembly mechanisms

Ryoma Ohi (University of Michigan, Ann Arbor, USA) discussed the observation that Eg5-independent bipolar spindle assembly occurs in nematodes, slime moulds and brown moss, but what about mammalian cells? It was previously observed that Eg5 inhibitors suppress spindle assembly in cell culture, but not in clinical settings. The kinesin motor Kif15 can rescue spindle assembly in cells that are challenged by Eg5 inhibitors suggesting that a Kif15-based pathway acts redundantly with Eg5. Therefore, a more effective therapeutic strategy is to inhibit both Eg5 and Kif15. He described efforts to screen for specific small-molecular inhibitors of Kif15 and a promising lead obtained by querying a kinase inhibitor library curated by GlaxoSmithKline (Dumas et al., 2019). Allen Leary (McGill University, Montreal, Canada) presented the contributions of a kinesin-5 to bipolar spindle formation that sequentially integrates crosslinking and sliding. Initially, in the monopolar spindle, the kinesin-5 crosslinks short MT pairs oriented at a high angle and mediates their reorientation in order to form a nascent bipolar spindle. Once spindle bipolarity is achieved, kinesin-5 sliding is required to stabilize spindle length fluctuations and set an equilibrium length (Leary et al., 2019 preprint). Renata Basto (Institut Curie, CNRS, Paris, France) discussed spindle assembly during neurogenesis. Primary recessive microcephaly, a condition that affects brain size without affecting body size, is a neuro-developmental disorder that results from mutations in centrosome or spindle-associated proteins. To unravel the underlying mechanisms, they examined spindle morphology in neural stem cells during brain development and found an increase in MT density from early to late stages of neurogenesis. At early stages, spindles contain more astral MTs, and later stages contain longer and thicker kMTs. These differences in spindle morphogenesis may allow for spindle orientation during symmetric divisions in early stages and might explain the vulnerability of neural stem cells to mutations affecting spindle morphogenesis.

Franz Meitinger (University of California, San Diego, USA) focused on mechanisms of acentrosomal spindle assembly. Retinal pigment epithelium 1 (RPE-1) cells treated with centrinone, an inhibitor of centrosome duplication, lose centrosomes during subsequent cell divisions. These cells divide and build a bipolar spindle, but eventually cell proliferation ceases. Using a CRISPR/Cas9 screen, Meitinger identified that knockout of tripartite motif-containing protein 37 (TRIM37) ubiquitin ligase allows acentrosomal cells to proliferate. TRIM37 is a negative regulator of polo-like kinase 4 (Plk4) self-assembly, which, in acentrosomal cells, creates ectopic foci for MT nucleation. In contrast, overexpression of TRIM37 leads to spindle assembly and chromosome segregation errors. Since TRIM37 is overexpressed in some cancer types this makes them particularly sensitive to centrinone treatment.

What drives centrosome movement and location? Jorge Ferreira [Instituto de Investigação e Inovação em Saúde (i3S) and IBMC, Porto, Portugal] discussed mechanisms of centrosome separation during early mitosis with a focus on dynein and the mechanical properties of the cell cortex and nuclear envelope. Ferreira presented a model in which in the early stages of centrosome separation cortical dynein orients the centrosomes along the main cell axis. As the cell rounds up, the cell cortex reorganizes, and dynein is loaded on to the nuclear envelope with an asymmetric distribution following the long nuclear axis, and this positions the centrosomes along the shortest nuclear axis. After nuclear envelope breakdown, dynein relocalizes back to the cell cortex and the spindle is reoriented to align with the main cell axis. Interestingly, the localization of dynein on the nuclear envelope is influenced by mechanical properties of the nucleus. Impairing chromosome condensation with a topoisomerase II inhibitor inhibits dynein loading on the nuclear envelope. In the same conditions, applying mechanical perturbation by squeezing the nucleus re-loads dynein onto the envelope. Patrick Meraldi (Medical Faculty of University of Geneva, Switzerland) raised the question of how do centrosomes influence k-fibre plus-ends that are six microns away. Inhibition of centrosome duplication leads to cells that assemble spindles with one centrosome. Such spindles are asymmetric with the half-spindle that lacks the centrosome being ∼50% shorter than the other half-spindle. Interestingly, the MTs of the shorter half-spindle are more stable and resistant to nocodazole treatment. He showed that depletion of hepatoma up-regulated protein (HURP), a protein that binds plus-ends of mitotic k-fibres, partially restores spindle symmetry in one-centrosome spindles and equalizes the MT stability of two half-spindles in nocodazole experiments. HURP is enriched on the plus-ends of shorter k-fibres and acts as a specific 'taxol', or stabilizer, for these fibres. This suggests that centrosomes could act indirectly on k-fibre length by determining HURP recruitment, thereby regulating spindle and cell division symmetry. It remains to be seen if this mechanism is implicated in naturally occurring asymmetric cell division (Dudka et al., 2019 preprint).

Regulation of microtubule size and stability in cells

MT regulation was discussed in a range of contexts throughout the meeting. Iain Cheeseman (Whitehead Institute for Biomedical Research and Massachusetts Institute of Technology, Cambridge, USA) described a CRISPR/Cas9-based approach to dissect mitotic spindle function and discover novel regulators. Cheeseman's group has developed an inducible knockout library for robust, specific and irreversible elimination of essential genes. Using this tool, they plan to address fundamental questions such as: what are the cellular consequences of anti-mitotic drugs? This is particularly interesting for drugs such as taxol and nocodazole, which have same overall effects on cell state but opposite effects on MT dynamics. This approach of combining the CRISPR/Cas9 screen with anti-mitotic drugs has the potential to reveal new functions of known proteins and uncover novel spindle regulators. Alexander Bird (MPI of Molecular Physiology, Dortmund, Germany) discussed a mechanism by which the clathrin protein stabilizes spindle MTs. Clathrin recruits G2 and S phase-expressed protein 1 (GTSE-1) to the spindle and k-fibres through adaptor-binding sites on its heavy chain, and loss of either protein causes a phenotype where chromosomes have trouble aligning at the metaphase plate. GTSE1 on the spindle, in turn, inhibits the activity of the MT depolymerase mitotic centromere-associated kinesin (MCAK) to promote MT stability and chromosome alignment. The use of a GTSE1 mutant that lacks clathrin binding activity revealed that the chromosome alignment phenotype is caused not by destabilization of k-fibres, but rather by astral MT destabilization, which provokes unsuccessful capture and congression of chromosomes. Francois Nedelec (University of Cambridge, Cambridge, UK) discussed their work on the MT marginal band in blood platelets. This ring of MTs pushes on the membrane and determines the discoid shape of the platelet. He presented new electron and super-resolution microscopy data challenging a long-standing model that the ring consists of a single stable MT that coils multiple times around the cell. Instead, the ring is composed of multiple MTs and the number scales with cell size. He further discussed how force in the marginal band could be produced by MT-crosslinking kinesins. He proposed that MTs in the marginal bands are composed of a mixture of newly polymerized tyrosinated and mature detyrosinated polymers. Crosslinking kinesins that walk with different speeds on adjacent, but differently modified MTs may result in marginal band expansion and produce a force against the cell membrane.

These talks highlight non-canonical mechanisms by which the architecture and mechanical properties of microtubule-based structures are regulated in cells. Screens designed to unearth new regulators promise to accelerate the discovery of novel mechanisms by which size and stability of cellular microtubules are tuned for specialized functions.

Theoretical and computational approaches to decipher spindle structure and function

Several groups discussed theoretical and computational approaches to address outstanding questions related to spindle assembly and function. Alex Mogilner (New York University, New York, USA) discussed a new model that expands on the traditional search-and-capture idea to explain the observed timeframe of spindle assembly. At prometaphase onset, centrosomal MTs pass through the kinetochore-containing area and form lateral attachments with a cloud of short MTs that are being nucleated at each kinetochore. The plus-ends of the short MTs are oriented towards the kinetochore by CENP-E. As the poles separate by Eg5 sliding, the kinetochores move only slightly to take on a torus-like conformation. When a threshold spindle length is achieved, the kinetochores acquire amphitelic attachments under dynein- and CENP-E-regulated mechanisms in a narrow time window. Meredith Betterton (University of Colorado, Boulder, USA) presented work on modelling spindle assembly in fission yeast. They consider the collective activity of motor and non-motor crosslinkers on MTs and ask what is needed to make a bipolar structure? Their work suggests that key functions of crosslinking proteins lead to a bipolar spindle: plus-end and minus-end directionalities of crosslinking motors Eg5 and kinesin14, respectively, and passive antiparallel crosslinking by anaphase spindle elongation protein 1 (Ase1) (Lamson et al., 2019). Interestingly, the model shows that the ability of Eg5 to switch directionality is crucial for spindle assembly. Agneza Bosilj (University of Zagreb, Zagreb, Croatia) presented a theoretical model that addresses how bridging MTs promote centring of the kinetochores. In this model, kinetochore positioning is navigated by kinesin-8 motors and antiparallel bridging MT overlap. Kinetochore fibres crosslinked by bridging MTs exert centring forces on kinetochores. When the bridging MT overlap is displaced from the spindle centre, kinetochores become re-centred around its new position. Furthermore, the presented model shows that positioning of the overlap in the centre of the spindle is a consequence of the kinesin-8 activity, whereas its length is regulated by passive cross-linkers, which enhance MT rescue frequency.

Elucidating the roles of the multiple mitotic kinesins in spindle assembly and function remains challenging due to redundancy and multi-tasking by the motors. The computation and theoretical approaches presented here reveal some of the key parameters that are necessary for accurately building the different substructures within the spindle at the right time and at the right place.

Concluding remarks

Bringing together colleagues with different approaches to study the spindle, a 'structure that we all love', as it was said by more than one presenter, allowed for many new ideas to be shared, new collaborations to be started and motivation to be reignited. All attendees were enthusiastic about reconvening and the second edition of this conference has been agreed upon and will be organized in Croatia in spring of 2021. We are looking forward to it!

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Competing interests
The authors declare no competing or financial interests.

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