A celebration of the 25th anniversary of chromatin-mediated spindle assembly

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ABSTRACT Formation of a bipolar spindle is required for the faithful segregation of chromosomes during cell division. Twenty-five years ago, a transformative insight into how bipolarity is achieved was provided by Rebecca Heald, Eric Karsenti, and colleagues in their landmark publication characterizing a chromatin-mediated spindle assembly pathway in which centromeres and kinetochores were dispensable. The discovery revealed that bipolar spindle assembly is a self-organizing process where microtubules, which possess an intrinsic polarity, polymerize around chromatin and become sorted by mitotic motors into a bipolar structure. On the 25th anniversary of this seminal paper, we discuss what was known before, what we have learned since, and what may lie ahead in understanding the bipolar spindle.

INTRODUCTION

The existence of a fusiform spindle apparatus that is wider in the middle and tapering at each end and its importance for accurate chromosome segregation had been hypothesized since cytologists such as Walther Flemming and Theodor Boveri peered through their microscopes in the late 19th century. In the 1940s, Shinya Inoué published polarized light microscopy videos of bipolar spindles in living plant and animal cells and demonstrated that the bipolar structure was composed of filaments that were in dynamic equilibrium with a soluble pool of subunits (Inoué, 1953, 1964). For decades thereafter, the question of how bipolar spindle morphology was achieved was an open one, until the “search and capture” hypothesis burst onto the scene in the 1980s (Kirschner and Mitchison, 1986). The model, which logically flowed from a rapid succession of landmark in vitro and cell-based studies, postulated that spindle morphology was defined by duplicated centromeres that nucleate microtubules (MTs) to search three-dimensional space until they capture a kinetochore and become stabilized (Mitchison and Kirschner, 1984a,b; 1985a,b; Mitchison et al., 1986). The hypothesis offered a simple and elegant molecular mechanism for the phenomenon of spindle bipolarity, in that its spatial cues came from two centrosomes searching for paired sister kinetochores. Twenty-five years ago, Heald et al. (1996) flipped the script by assembling bipolar spindles without centrosomes (search) or kinetochores (capture).

THE BEGINNING OF SPINDLE ASSEMBLY: SEARCH AND CAPTURE

Major advances in our understanding of the molecular composition of the spindle and the biochemistry of its constituents were made in the decades following Inoué’s polarized light microscopy–based characterization of spindle assembly. In the 1960s, Gary Borisy, as a student in Ed Taylor’s lab at the University of Chicago, used the drug colchicine, which had previously been shown by Inoué to disassemble bipolar spindles (Inoué, 1952), to biochemically isolate the colchicine-binding protein tubulin, the building block of the spindle filaments (Borisy and Taylor, 1967). It was subsequently shown that the subunit of the MT is a constitutive heterodimer of two tubulins called α and β tubulin (Bryan and Wilson, 1971; Ludueña et al., 1977). The MT possesses intrinsic structural polarity, since heterodimers assemble in a head-to-tail arrangement as protofilaments (typically 13 in vivo) that assemble via lateral interactions into a hollow tube (Nogales et al., 1998; Nogales et al., 1999). It was first shown in vitro and later confirmed in cells that one end of the MT (the plus-end) is more dynamic (faster-growing and faster-shortening) than the other end (the minus-end) and that the filament exhibits a striking steady state behavior called dynamic instability where its ends stochastically transition between growing and shortening (Mitchison and Kirschner, 1984a; Cassimeris et al., 1988; Walker et al., 1988).
Kinetochore motors are macromolecular machines that assemble at sister centromeres during cell division. The kinetochore has two essential functions: 1) mediating load-bearing attachments between dynamic spindle MTs and centromeres to move chromosomes, and 2) coordinating a biochemical pathway called the spindle assembly checkpoint (SAC) to delay anaphase onset until all the chromosomes are correctly attached to the spindle MTs (Musacchio andDesai, 2017). When Heald andKarsenti published their work in 1996, both functions were largely understood at the phenomenological level and characterization of the molecular mechanisms of attachment and SAC regulation was in its early stages. It had been convincingly established that unattached kinetochores were the source of the wait-anaphase signal (Rieder et al., 1994; Rieder et al., 1995). The core checkpoint proteins had been identified in genetic screens in budding yeast (Hoyt et al., 1991; Li andMurray, 1991), but the observation that one of these checkpoint proteins (Mad2) localized to unattached vertebrate kinetochores would be published several months after the description of chromatin-mediated spindle assembly by Heald et al. (1996; Chen et al., 1996; Li andBenezra, 1996). While the identity of centromere proteins (CENP-A, B, C) were known (Earnshaw andRothfield, 1985), the attachment factors of the outer kinetochore had not yet been characterized although it had been established in vitro and in cells that kinetochores could capture, stabilize, and even move on MTs (Mitchison andKirschner, 1985b; Mitchison et al., 1986; Hyman andMitchison, 1991).

Research on the molecular basis of attachment and chromosome movement at that time focused on MT-based motors, since dynein, CENP-E, and MCAK had all been shown to localize to mammalian kinetochores by immunofluorescence (Pfarr et al., 1990; Steuer et al., 1990; Yen et al., 1991, 1992; Wordeman andMitchison, 1995). The kinetochore localization of CENP-E and dynein was consistent with observations of poleward kinetochore-based motility along MTs in cells and bidirectional movement of kinetochores on MTs in vitro, although the plus-end directionality of CENP-E was not established until 1997—the year after Heald et al. was published (Merdes andDeMey, 1990; Rieder andAlexander, 1990; Hyman andMitchison, 1991; Wood et al., 1997). Beyond the kinetochore, a greater appreciation for the roles of mitotic motors in organizing the spindle was emerging in 1996 (Hyman andKarsenti, 1996). The initial study localizing dynein to vertebrate kinetochores also reported that the motor localized to centrosomes and spindle poles. Chromosome-associated kinesins (chromokinesins) had just been discovered (Afshar et al., 1995; Vernos et al., 1995; Wang andAdler, 1995); the minus-end directed motor Ndc (Walker et al., 1990) had been found two years before to be required for spindle pole organization in Drosophila melanogaster (Endow et al., 1994). Importantly, it was discovered 6 months before Heald et al. (1996) that disruption of the dynactin (dynein regulatory) complex caused severe spindle assembly defects in mammalian cells, leading the authors to hypothesize that dynein focused microtubule minus ends into the spindle poles (Echeverri et al., 1996).

While it was beginning to become evident that mitotic motors were contributing to spindle morphology, in 1996 centromeres were still thought to provide the dominant spatial cues for achieving spindle bipolarity. In addition to their description of MT dynamic instability in 1984, an accompanying paper by Mitchison and Kirchner showed that centrosomes nucleated MTs in vitro (Mitchison andKirschner, 1984b). A year later they reconstituted the capture of MT ends by kinetochores in vitro (Mitchison andKirschner, 1985b) and then demonstrated that kinetochores stabilized MTs in cells (Mitchison et al., 1986). The culmination of this body of work was the articulation of the search and capture model of spindle morphogenesis whereby astral MTs nucleated from centrosomes search for kinetochores in three-dimensional space via dynamic instability and become stabilized once they are captured by kinetochores (Kirschner andMitchison, 1986). The search and capture hypothesis was further bolstered when Rieder and colleagues visualized astral MT capture by kinetochores in living cells, although the kinetochores bound to the sides rather than to the ends of the MTs (Hayden et al., 1990; Rieder andAlexander, 1990).

**BIPOLAR SPINDLE ASSEMBLY WITHOUT SEARCH AND CAPTURE: THE DISCOVERY OF CHROMATIN-MEDIATED ASSEMBLY**

It is noteworthy that some of the first videos of bipolar spindles in living cells were taken of pollen cells from the Easter lily (Inoué, 1953, 1964) because plants do not have centrosomes. Similarly, female meiotic cells in most animals lack centrosomes, yet assemble bipolar structures (Dumont andDesai, 2012). Thus, while search and capture applied nicely to spindles in animal somatic cells, it was not meant to explain spindle bipolarity universally. In the 1980s, Karsenti and colleagues showed that injection of high-molecular weight DNA (lacking any centromeric sequences) into metaphase-arrested Xenopus laevis eggs promoted MT assembly (Karsenti et al., 1984). A decade later, Rebecca Heald began coupling linearized and biotinylated “Bluescript plasmid containing a 5-kb insert of non-coding Drosophila DNA” to streptavidin-coated magnetic beads to build “artificial chromosomes” lacking kinetochores in X. laevis egg extracts (Heald et al., 1996). Mitotic chromatin could be assembled on the beads by cycling them, via addition of Ca2+ to the extract, through interphase and then adding back fresh metaphase-arrested egg extract. During interphase the DNA was replicated and the chromatin beads assembled functional nuclei with a double membrane and nuclear lamina that supported nuclear transport (Heald et al., 1996). As Heald describes it, after using the mitotic chromatin-coated beads for some “horrible” radioactive phosphatase activity assays, she decided to spike some fluorescently labeled tubulin into the extract and “look at them instead” under the microscope (Rebecca Heald, personal communication). The first glimpse through the eyepiece must have been a “Eureka!” moment, for the chromatin-coated beads assembled beautiful bipolar spindles that were morphologically indistinguishable from spindles assembled in the extract using replicated sperm chromosomes that possessed kinetochores and centrosomes (Heald et al., 1996). Because the DNA beads did not contain centromeric sequences and the Xenopus egg extract did not have centrosomes, the major driving force for bipolar spindle assembly in this assay was the chromatin itself (Heald et al., 1996). Today, this pathway is referred to as chromatin-mediated spindle assembly (Supplemental Video 1).

A more detailed investigation into the stages of chromatin-mediated spindle assembly revealed that the process depends on self-organization of MTs into a bipolar array (Figure 1). Randomly oriented MTs were nucleated in the vicinity of the beads in the first 15–30 min, after which they began to “coallesce” into antiparallel bundles before becoming focused into spindle poles that extended away from the beads into a bipolar structure after 60–90 min. Interestingly, polarity-marked MT seeds within the bead spindles moved poleward, with their minus-ends leading in a dynein-dependent manner, and dynein activity was shown to be required for the establishment and maintenance of focused bead spindle poles (Heald et al., 1996). Heald and colleagues concluded the paper with a new model for spindle assembly in which they proposed that microtubules are self-organized into a bipolar structure by the combined activities of plus- and minus-end directed motors acting upon the randomly oriented array that is nucleated in the vicinity of the mitotic.
The spindle poles away from the beads. The minus-end directed motors dynein and XCTK2 (kinesin-14) contributed to pole focusing. Thus, multiple motors contribute to the self-organization phenomenon and, in many cases, these motor functions are conserved in somatic cell spindle assembly.

Chromatin-mediated pathways function in somatic animal cells
The skeptics’ argument that the chromatin-mediated pathway was limited to plant and female meiotic cells was silenced by a series of studies over the next decade. First, it was shown that functional bipolar spindles assembled normally following laser ablation of one or both centrosomes in vertebrate somatic tissue culture cells (Khodjakov et al., 2000). It was later shown that centrosomes were dispensable for spindle bipolarity in various mutants of D. melanogaster that lacked centrosomes (Bonaccorsi et al., 2000; Giansanti et al., 2001; Megraw et al., 2001; Basto et al., 2006). The hypothesis in Heald et al. that “the real function” of centrosomes was to regulate spindle orientation/positioning by linking the spindle poles to the cell cortex has been supported by years of subsequent research.

Chromatin-based signals for MT assembly
At the conclusion of their work, Heald and colleagues noted that they did not know how chromatin induced MT assembly, but they favored the explanation that the mitotic chromatin locally altered the state of the cytoplasm to promote nucleation and stabilization. This hypothesis fueled much research in the field over the subsequent 25 years, which led to our present-day understanding that spatial gradients around mitotic chromatin promote MT polymerization. A gradient of RanGTP, which is generated by its chromatin-associated GEF RCC1, triggers the local release of spindle assembly factor (SAF) from import receptors around mitotic chromatin (Carazo-Salas et al., 1999; Kalab et al., 1999; Ohba et al., 1999; Wilde and Zheng, 1999; Zhang et al., 1999; Carazo-Salas et al., 2001; Gruss et al., 2001; Nachury et al., 2001; Wiese et al., 2001; Kalab et al., 2002; Kalab et al., 2006; Kalab and Heald, 2008; Halpin et al., 2011). There are many SAFs targeted by the RanGTP gradient, but, at present, the SAF with the most direct link to MT nucleation is TPX2, since it binds to and activates Aurora A kinase, which

FIGURE 1: A comparison of two models of spindle assembly: search and capture versus self-organization. (A) In the classic search and capture model, the spindle axis is predetermined by centrosomes, which are positioned on either side of the nucleus during prophase. After the nuclear envelope breaks down in prometaphase, centrosome-nucleated MTs (green) search the three-dimensional volume of the cell via dynamic instability until they physically contact a kinetochore (blue) and become captured and stabilized. The attachment of MTs from opposite centrosomes to the two sister kinetochores on a chromosome (orange) coupled with a balance of forces leads to its central positioning between the two centrosomes—culminating with the alignment of every pair of sister chromatids at a metaphase plate. (B) In the self-organization model (shown here around chromatin beads [orange]), randomly oriented MTs (green) assemble in the vicinity of mitotic chromatin due to localized activity gradients. The spindle axis is defined when the MTs are sorted (often into antiparallel arrays) and coalesced into bundles by the actions of plus-end directed motor proteins, including the tetrameric kinesin-5 and chromokinesins, which also drive extension of the MTs away from the DNA. The minus-ends of the MTs are clustered and focused into spindle poles by the minus-end directed motors dynein or kinesin-14. During bead spindle assembly these “steps” are occurring concomitantly and, once assembled, they are required to continuously maintain the fusiform structure, as inhibiting molecular mediators of nucleation (e.g., Ran pathway), coalescence and pole extension (e.g., kinesin-5), or pole focusing (e.g., dynein) affects the morphology of an assembled bipolar spindle.
in turn phosphorylates NEDD1 to promote γ-TuRC-mediated MT nucleation (Scrofani et al., 2015). Interestingly, the chromosomal passenger complex (CPC) and its constituent Aurora B kinase are also an important regulator of MT assembly around chromosomes through local inhibition of the catastrophe factors MCAK and Stathmin/Op18 (Andersen et al., 1997; Andrews et al., 2004; Sampath et al., 2004; Gadea and Ruderman, 2006; Kelly et al., 2007; Maresca et al., 2009). In this case, the activity gradient promotes MT stability, since it reduces the catastrophe frequency of polymerizing MTs in the vicinity of mitotic chromatin. Thus, regarding their hypothesis about the nature of the MT assembly signal around chromatin, Heald and colleagues were prescient in the 1996 paper.

WHAT LIES AHEAD

Self-organizational processes are prevalent and function on multiple scales during spindle assembly. The significance of assembling bead spindles in the absence of kinetochores was rightly emphasized by Heald and colleagues in 1996. However, like micrometer-sized DNA-coated beads, nanometer-scale kinetochores also nucleate and organize MTs (Telzer et al., 1975; Mitchison and Kirschner, 1985a; Khodjakov et al., 2003; Maiato et al., 2004). In fact, kinetochores nucleate randomly oriented MTs that are coalesced into a bundle and extended away from the chromosome with MT minus-ends oriented away from the kinetochore—a phenomenon that is remarkably similar to Heald’s description of the steps of chromatin-mediated spindle assembly. The kinetochore-mediated process also utilizes molecules that are central to chromatin-mediated spindle assembly: import receptors, TPX2, dynemin, and the CPC (Tulu et al., 2006). Furthermore, sorting of randomly oriented MTs around kinetochores during coalescence is mediated by the plus-end-directed kinetochore-associated motor protein CENP-E (Skirzhetsky et al., 2018).

Like chromatin-mediated spindle assembly, the kinetochore-mediated pathway appears to be as far from the classic search and capture model as one could imagine—at least in its early stages. Mathematical modeling revealed that search and capture that relies on dynamic MTs nucleated from centromeres could not work in a human cell with physiologically relevant timing unless it was spatially biased (Wollman et al., 2005). It is now known that there is a noncentrosomal MT nucleation pathway that structurally biases the direction of MT growth. Branching MT nucleation is mediated by recruitment of the γ-TuRC to a MT by the augmin complex (Goshima et al., 2007; Uehara et al., 2009; Petry et al., 2013; Verma and Maresca, 2019; Alfaro-Aco et al., 2020; Tariq et al., 2020). Importantly, daughter MTs are nucleated so that their growing plus-ends are oriented in the same direction as the mother MT.

Most spindle MTs do not persist long enough to support branching nucleation, since the multistep process takes ~30s (Verma and Maresca, 2019). However, during kinetochore-mediated assembly, CENP-E sorts MTs and converts them from lateral interactions into end-on attachments where the MT plus-ends are inserted into the kinetochore and stabilized, at which point they will live long enough to support branching. At this stage, daughter MTs nucleated via branching will 1) be closer to the kinetochore than if they were nucleated by the centromere, and 2) grow with their plus-ends oriented in the direction of the kinetochore. Thus, while self-organization mechanisms initially organize kinetochore MTs, the mature kinetochore fiber is likely assembled with a biased search and capture mechanism that relies on branching (rather than centrosomal) MT nucleation (Goshima et al., 2008; Uehara et al., 2009; Kamasaki et al., 2013; David et al., 2019; Verma and Maresca, 2019; Almeida et al., 2021). As more is learned about how functional bipolar spindle assembly, we should not be surprised to discover that both biased search and capture and self-organization mechanisms contribute to building this incredibly complex machine—it may just take slightly altering your perspective to observe when and where they are at work.

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