Mitotic spindle membranes

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ABSTRACT **The mitotic spindle, which uses microtubules (MTs) and MT-based motor proteins to separate sister chromosomes prior to cell division, contains abundant membranes, organelles, and protein assemblies derived from the familiar interphase intracellular membrane network. In this essay, mainly with reference to selected animal and fungal cells, I summarize current ideas about the reciprocal functional relationship between these mitotic spindle-associated membranes and the spindle MT cytoskeleton, in which; 1) spindle membranes control the composition, Ca^{++} ion concentration and mechanical performance of the spindle MT cytoskeleton; and conversely 2) the spindle MT cytoskeleton contributes to membrane/organelle partitioning and inheritance during cell division and serves as a reservoir of membranes, organelles, and vesicles for delivery to the interphase cytoplasm, plasma membrane, and cleavage furrow.**

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INTRODUCTION

The mitotic spindle is a bipolar, microtubule (MT)-based machine that assembles itself; captures, aligns, and separates sister chromosomes; and then positions the cleavage furrow in preparation for cell division (Inoué and Salmon,1995; Scholey *et al.*, 2003; Drechsler and McAinsh, 2012; McIntosh *et al.*, 2012; Pollard and O'Shaughnessy, 2019; Valdez *et al.*, 2023). This process depends upon forces generated by multiple molecular motors, mainly kinesins, dyneins, and dynamic MT polymer ratchets, whose activities are controlled by cell cycle kinases (notably cyclin-dependent, aurora, and polo-like kinases) phosphatases (PP1 and PP2A) and proteases. Mitotic spindles (herein meaning the entire mitotic apparatus including astral as well as interpolar and kinetochore MTs) also have several types of associated membranous elements derived from the interphase membrane network (Figures 1 and 2). The structure and dynamics of these spindle-associated membranes were characterized early on by electron micsoscopy (EM) (Hepler and Wolniak, 1984) and subsequently by live cell imaging (Terasaki and Jaffe, 1991; Waterman-Storer *et al.*, 1993). What is the origin, nature, and cell biological significance of mitotic spindle membranes? To address this question in this fascinating, complex, evolving field, here I outline; (i) the diversity of spindle-associated membranous elements in various cells; (ii) membranes as barriers or scaffolds that control spindle composition; (iii) membrane control of spindle Ca^{++} concentration; (iv) the contribution of membranes and membrane-derived protein assemblies to spindle mechanics; (v) spindle-mediated partitioning of membranous structures; and (vi) the delivery of partitioned spindle membranes to the cytoplasm, cell membrane, and cleavage furrow.

(i): Membranes are a common feature of mitotic spindles and display system-specific variations in their identity, morphology, and dynamics

It is evident that the mitotic spindle often performs its function in a close spatial relationship with the endoplasmic reticulum (ER) and nuclear envelope (NE), which together form a membranous continuum within eukaryotic cells. The NE is a differentiated subdomain of the dynamic interphase ER network, whose double lipid bilayer

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Abbreviations used: aMTs, astral Mts; BuGZ, Bub3 interacting and GLEBs containing ZNF207 protein; BDM, 2,3-butanedione monoxime; chTOG, colon and hepatic TOG protein; EB1, end-binding protein 1; EM, electron microscopy; ER, endoplasmic reticulum; GFP, green fluorescent protein; GM130, golgi matrix protein 130; IC, intermediate compartment; InsP3, inositol 1,4,5-trisphosphate; ipMT, interpolar MT; kMT, kinetochore MT; LECA, last eukaryote common ancestor; LLPS, liquid-liquid phase separation; MT, microtubule; MTOC, microtubule organizing center; NE, nuclear envelope; NEB, nuclear envelope breakdown; NL, nuclear lamina; NM, nuclear membrane; NPC, nuclear pore complex; NuMA, nuclear mitotic apparatus protein; PM, plasma membrane; PP, protein phosphatase; SAF, spindle assembly factor; TPX2, Targeting protein for Xklp2 (a kinesin-12); TACC3, Transforming acidic coiled coil-containing protein 3; TGN, Trans golgi network; TOG, Tumor over expression gene domain protein.

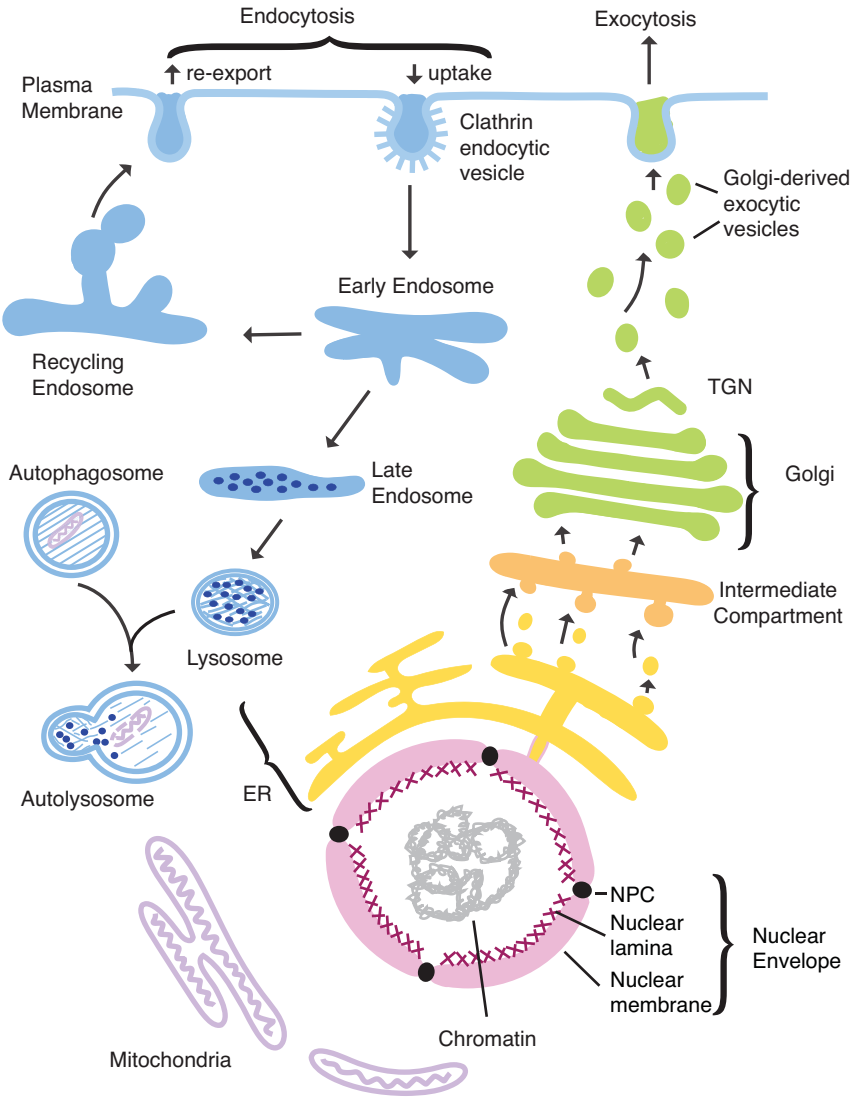

FIGURE 1: Schematic cartoon depicting components of the interphase membrane network that associate with mitotic spindles (see Figure 2) within a hypothetical, generic eukaryotic cell. The interphase membrane network includes the endomembrane system, whose compartments are linked by vesicular trafficking, comprising the NE (i.e., the NM plus NPCs and NL) which forms a continuum with the ER (Deolal *et al.*, 2024; Kors and Schlaitz, 2024); the Golgi apparatus with associated intermediate compartment, trans Golgi network (TGN) and exocytic vesicles; clathrin-coated endocytic vesicles, maturating early, late, and recycling endosomes with associated recycling vesicles (where the balance between uptake and re-export controls plasma membrane [PM] composition); lysosomes and autophagosomes; and the PM (Carlton *et al.*, 2020). In addition, it includes mitochondria, peroxisomes (not shown) and in plant cells plastids such as chloroplasts (not shown).

nuclear membrane (NM) is characterized by the presence of nuclear pore complexes (NPC) and the nuclear lamina (NL; composed of intermediate filament proteins, lamins A, B, and C and generally considered to be specific to metazoa) (Figure 1). Throughout the eukaryote domain, there exists system-specific variation in the extent to which the NE remains intact or disassembles during mitosis and generates membrane-derived structures, notably protein assemblies, that associate with the spindle and contribute to its function (Zheng, 2010; Dey and Baum, 2021) (Figure 3).

For example, many protists and fungi, such as *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae*, and *Aspergillus nidulans* (De Souza and Osmani, 2007; Mori and Oliferenko, 2020; Dey and Baum, 2021) and possibly also LECA (the syncytial last common ancestor of eukaryotic cells; Bremer *et al.*, 2023), undergo so-called "closed" mitoses (Figure 3(i)) in which the NE remains largely intact with only transient, localized disassembly of the NM and NPC occurring. In contrast, during prophase of the "open" mitoses of for example echinoderm embryos and vertebrate somatic cells (Figure. 3(iii)), disassembly of the NE is almost complete, requiring extensive disassembly of the NM, NPCs, and NL (Ellenberg *et al.*, 1997; Terasaki *et al.*, 2001; Deolal *et al.*, 2024). Here, nuclear envelope breakdown (NEB) is cell cycle–regulated and involves the solubilization of nuclear lamins A and C, whereas lamin B may remain associated with NE/ER membranes or cytoplasmic vesicles or instead form a "membranous lamin B spindle matrix" (Tsai *et al.*, 2006; Zheng, 2010). (A spindle matrix is

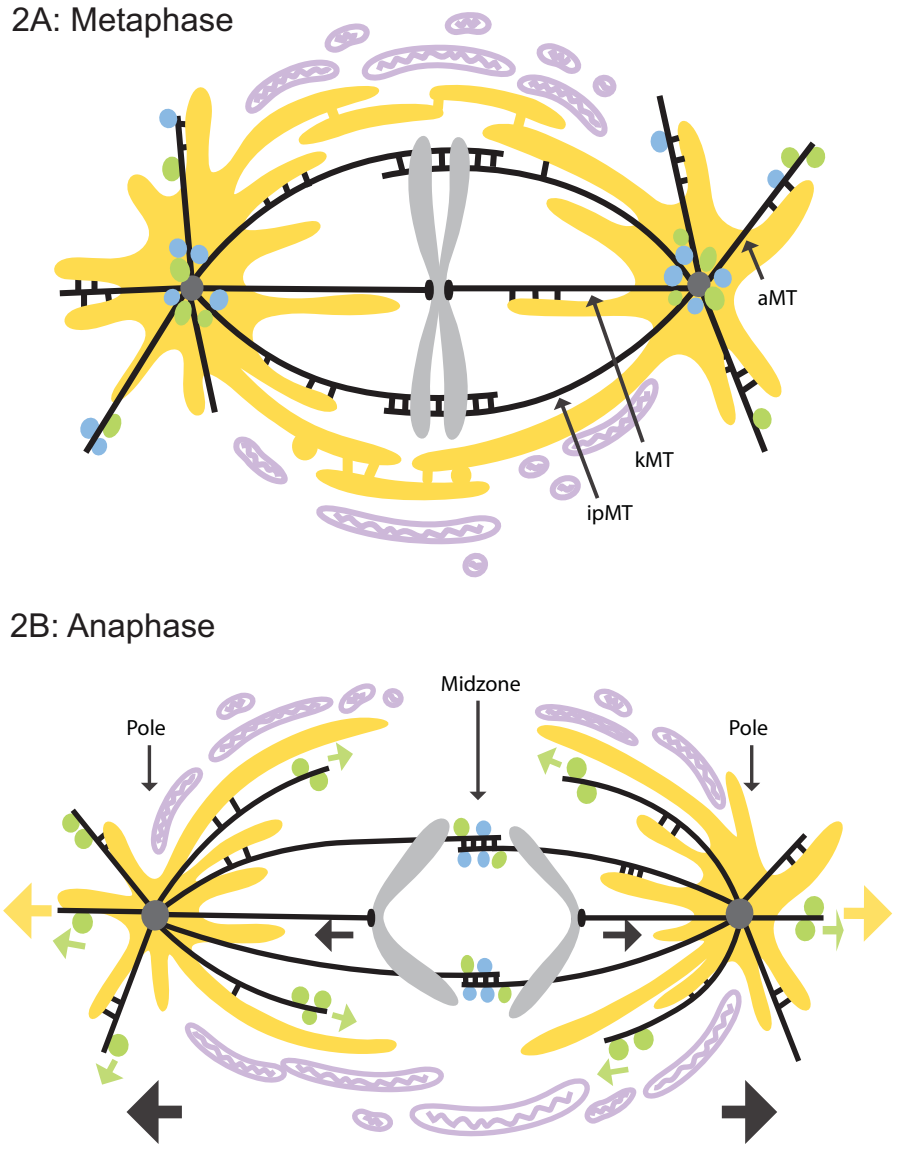

FIGURE 2: Schematic cartoon depicting intracellular membrane components that associate with the mitotic spindle in a hypothetical generic eukaryotic cell. During M-phase, the membranous compartments of an interphase cell (Figure 1) undergo a striking dynamic reorganization and redistribution to the daughter cell products of cell division, where they reform (Carlton *et al.*, 2020). As depicted here, using the same color code as in Figure 1, subpopulations of these membranes associate with the mitotic spindle (Hepler and Wolniak, 1984). Thus, by metaphase (A), the NE and Golgi have merged into the ER (Kors and Schlaitz, 2024) to form an ER/NE/Golgi continuum shown in yellow that associates with the spindle, and is surrounded by a layered mitochondrial network (purple). Exocytic vesicles (green) and endosomes (blue) cluster around the poles and along aMTs. A gray chromosome comprising two sister chromatids lies at the equator. In the oversimplified spindle cartoon, black kinetochore microtubule (kMT), ipMT, and astral microtubule (aMT) denote kinetochore, interpolar, and astral MTs, respectively. A few black MT-MT and MT-ER cross-links are indicated. During anaphase (B), chromosome-to-pole motility and pole-to-pole separation are denoted by thick black arrows. Spindle elongation-mediated partitioning of the ER/NE/Golgi network (e.g., Henson *et al.*, 1989; Terasaki, 2000) is denoted by outward pointing yellow arrows. The motor-driven transport of exocytic vesicles along aMTs toward the cell surface and equator for exocytic membrane repair and cytokinetic membrane expansion is indicated by green arrows (Bi *et al.*, 1997; Shuster and Burgess, 2002). Green exocytic and blue recycling endosome membranes have translocated onto the midzone for subsequent delivery to the ingressing cleavage furrow.

any non-MT component of the spindle that complements the action of MTs and motors, to control spindle morphogenesis and function [Johansen and Johansen, 2007; Yao *et al.*, 2012]). During late stages of open mitosis, the NE starts to reform on the surface of separated sister chromosomes thereby initiating complete NE reassembly (Deolal *et al.*, 2024; see El Mossadeq *et al.*, 2025 for an interesting meiosis-associated variant example of this process). In between these extremes, there are "semi-open" forms of mitosis in which the NE disassembles during only part of mitosis for example in the *Caenorhabditis elegans* (Hayashi *et al.*, 2012) and *Drosophila* syncytial (Stafstrom and Staehelin, 1984; Smyth *et al.*, 2015) embryos (Figure 3(ii)). In the latter system, for example, the

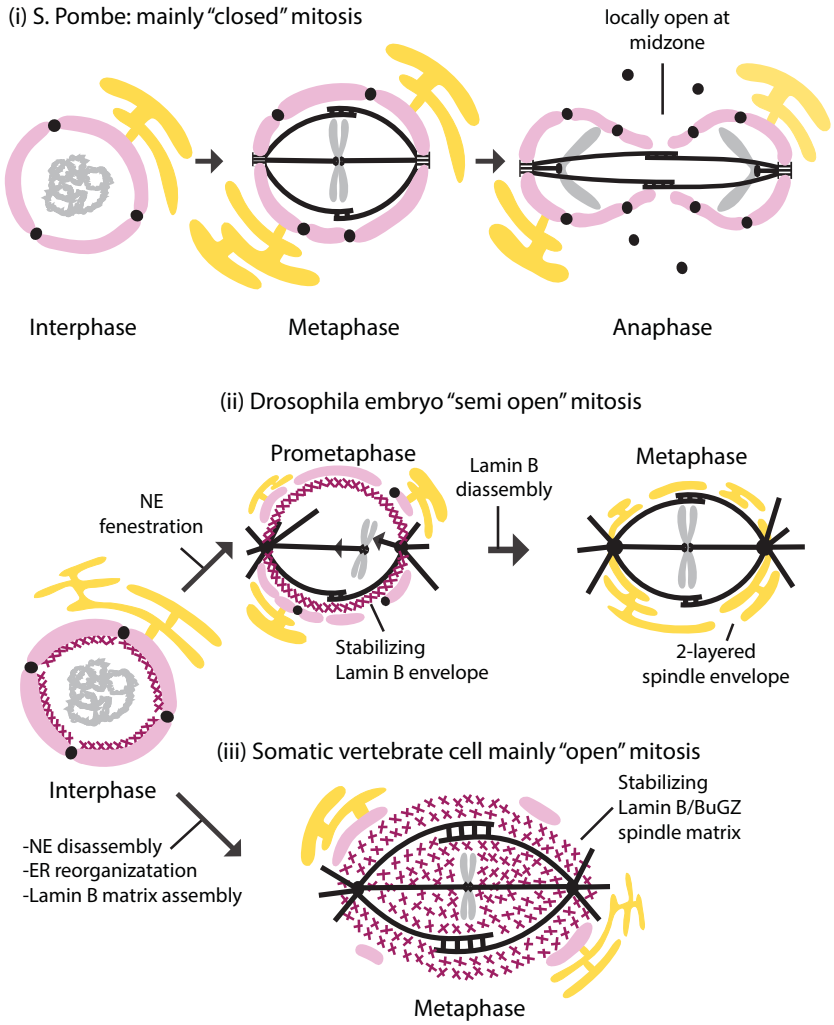

FIGURE 3: Schematic cartoons depicting spindle envelopes and mechanical scaffolds associated with mitotic spindles. For simplicity, in this figure the ER (yellow) NM (pink) and nuclear lamin B (purple x) are depicted as separate structures, although, as noted in Figures 1 and 2, the spindle envelope is best considered as comprising an ER/NE/Golgi network. Although it is customary (and convenient) to classify spindles as (i) "closed", (ii) "semi-open" and (iii) "open", it is currently argued that the extremes of completely open and completely closed configurations do not exist, but instead there is a continuum between "mainly closed" (e.g., *S.pombe* (i)) and "mainly open" (e.g., somatic mammalian cell (iii)) configurations (Dey and Baum, 2021). The B-type lamins are absent in fission yeast (i). In contrast they form a spindle-stabilizing, disassembling lamin B envelope around *Drosophila* embryo prometaphase spindles following NEB at the poles (ii). The precise dynamic interrelationships between this lamin B envelope, the residual, fenestrated NE and the ER spindle envelope, which is fully intact by metaphase, are unclear. Arrowheads in the prometaphase cartoon denote a MT pushing a congressing chromosome arm and kMT pulling its kinetochore towards the equator. (iii) Lamin B assembles into a "membranous" lamin B/BuGZ matrix that is a core structural component of the spindles of some vertebrate cells. The lamin B protein assemblies depicted in panels (ii) and (iii) are proposed to interact functionally with mitotic motors and nonmotor SAFs to facilitate spindle assembly, maintenance and function (see text). Only the spindle regions are shown in this Figure.

prophase NE is deformed by centrosome-nucleated aMTs until it eventually ruptures at the poles, allowing these MTs and associated spindle assembly factors (SAFs) to invade the nuclear space. A lamin B envelope, which persists throughout prometaphase, stabilizes the assembling bipolar spindle as chromosomes are captured within the increasingly fenestrated residual NE. Subsequently, as the lamin B envelope disassembles, the ER assembles into a two-layered, fenestrated "spindle envelope" that persists throughout metaphase and anaphase (Figure 3(ii)) (Stafstrom and Staehelin, 1984; Civelekoglu-Scholey *et al.*, 2010; Smyth *et al.*, 2015).

A portion of the extensive cytoplasmic ER network also associates with the spindles of many protist, plant and animal cells during mitosis. Spindle-associated ER membranes often concentrate around the poles during prophase then reorganize into various system-specific morphologies. For example, as already noted, fenestrated ER lamellae form a "spindle envelope" in *Drosophila* embryos, and also in neuroblasts and spermatocytes (Stafstrom and Staehelin, 1984; Smyth *et al.*,2015). In echinoderm embryo spindles, ER membranes concentrate around the spindle poles and generate tubules that radially disperse along astral MTs and along

metaphase and anaphase half spindle MTs but not spindle midzone MTs from which they are excluded (Harris, 1975; Henson *et al.*, 1989; Terasaki and Jaffe, 1991) (Figure 2, A and B). In mitotic mammalian cells, the bulk of the ER continuum remains intact, lying outside the spindle toward the cell periphery (Ellenberg *et al.*, 1997; Puhka *et al.*, 2007). However, in contrast to the prevalent opinion that metaphase half-spindles are membrane-free, ER membranes also localize to mammalian cell mitotic spindles (Waterman-Storer *et al.*, 1993; Lu *et al.*, 2009; Puhka *et al.*, 2012; Schlaitz *et al.*, 2013; Zhao *et al.*, 2023). An excellent recent review of ER remodeling during mitosis complements the current review by detailing mitosis-associated changes in the ER's morphology and metabolic functions; its uptake of NE and Golgi components; and its association with MTs, actin and intermediate filaments, topics that are not covered in detail here (Kors and Schlaitz, 2024).

Several other components of the endomembrane system associate with mitotic spindles in various cell-types (Figures 1 and 2), as discussed in more detail later (Section (v)). For example, the dynamic interphase Golgi apparatus (Cole *et al.*, 1996) is proposed to reorganize in two ways. First it may disassemble into vesicles, some of which concentrate around the spindle poles before reassembling during telophase (Ayala and Colanzi, 2022); and second, a portion of the cell's Golgi apparatus may merge into the spindle-associated ER, as in sea urchin blastomeres for example (Terasaki, 2000). Lysosomes often disperse during early mitosis then recluster at the spindle poles during telophase, but in between a subset may associate with mitotic chromosomes (Hämälistö *et al.*, 2021). Energy-generating mitochondria are generally excluded from mitotic spindles but often cluster around the spindle periphery, forming a layered boundary between the spindle and cytoplasm (Hepler and Wolniak,1984) (Figure 2A).

Thus, it is common to find membranous structures associated with mitotic spindles, where they appear as boundaries that separate the mitotic spindle from the surrounding cytoplasm or localize to specific regions within the spindle, and it is reasonable to hypothesize that these associations are biologically significant. However, it may be prudent to bear in mind the caveat that nonphysiological objects such as microinjected beads can also accumulate in mitotic spindles but whether they do so nonspecifically or by mimicking physiological cargo is not always established (Wadsworth, 1987). Consequently, there are many unresolved questions in this interesting field.

(ii): Membranes as barriers or scaffolds that control spindle composition

During closed and semi-open mitoses, a membranous spindle envelope serves as a transport barrier that selectively controls the composition of the spindle domain to facilitate mitotic progression (Dey and Baum 2021) (Figure 3). In *Drosophila* S2 cells, for example, a spindle envelope derived from the NE/ER network forms an organelle exclusion barrier that uses a MT-independent molecular crowding mechanism for the selective accumulation of molecules within the spindle domain (Schweizer *et al.*, 2015) (Figure 3(ii)). It is proposed that space limitations due to the large volume occupied by cytoplasmic organelles forces diffusible molecules, for example, tubulin subunits, into the relatively uncrowded nuclear domain, where spindle assembly is promoted. In the closed mitosis of *S. pombe*, the intact NE serves as a selective transport barrier between spindle and cytoplasm throughout much of mitosis, with its component spindle pole body directing prophase spindle assembly (Figure 3 (i)). However, the transient, localized opening of the NE at the midzone region is required to facilitate the final steps of nuclear division. To accomplish this, the central domain of the NE closely juxtaposes with the narrow internuclear bridge at the spindle midzone and undergoes compartmentalized NPC and localized NM disassembly, leading to complete nuclear fission and the formation of two daughter nuclei (Dey *et al.*, 2020).

The sequence of events associated with transient NE opening at the midzone in *S. pombe* resembles NEB in the open mitosis of echinoderm oocytes which proceeds in steps of (i) NPC disassembly; (ii) NM fenestration; and finally; (iii) completion of NE disassembly (Lénárt *et al.*, 2003). In such open mitoses, including those of many somatic vertebrate cells, the selective barrier function of the disassembled NE appears to be absent during most of mitosis. However, in their paper on the spindle envelope of fly S2 cells, Schweizer *et al.*, 2015 propose that human cells contain a similar ER-derived spindle envelope that serves as an organelle-exclusion barrier and controls spindle composition mainly during early mitosis. Furthermore, it is plausible to think that the NE-derived lamin B matrix proposed by Zheng and colleagues (Figure 3(iii)) may play an analogous role to a membranous transport barrier in cells undergoing open mitoses for example by selectively binding and sequestering soluble proteins within the spindle domain using a diffusion-to-capture mechanism (Tsai *et al.*, 2006; Zheng, 2010).

(iii): Membrane control of Ca^{++} concentration in the spindle

A special case of membranes controlling spindle composition concerns Ca^{++} regulation by the spindle ER network. Early on it was noted that this network resembles the sarcoplasmic reticulum, which mediates Ca^{++} regulation of striated muscle contraction via troponin-tropomyosin and myosin regulatory light chains. This led to the hypothesis that; (i) Ca^{++} transport ATPases could pump Ca^{++} into the ER to maintain a low level of 10^{-7} M Ca^{++} in the spindle; and (ii) the release of Ca^{++} from the ER to a level of 10^{-6} M at the metaphase-anaphase transition could activate calmodulin-like regulatory EF-hand proteins, which in turn could bind and activate anaphase motors to drive chromosome-to-pole motility (Hepler and Wolniak, 1984).

In support of this hypothesis, echinoderm embryo spindles were found to contain both a Ca^{++} transport protein and a Ca^{++}-binding protein, calsequestrin, that could cooperate to transport Ca^{++} into the lumen of the mitotic spindle–associated ER and sequester it there (Petzelt and Hafner, 1986; Henson *et al.*, 1989). Moreover, Ca^{++} sequestration and release by this system could contribute to the series of transient Ca^{++} spikes observed using fluorescent reporters during mitosis of the first cell cycle for example at NEB and anaphase onset (Poenie *et al.*, 1985; Steinhardt and Alderton, 1988). Significantly, the experimental elevation of spindle Ca^{++} concentrations to > micromolar levels, either by direct Ca^{++} microinjection or by inhibition of the Ca^{++} importing system with caffeine or antibody microinjection, led to the disassembly of mitotic spindle MTs and mitotic arrest (Kiehart, 1981; Hafner and Petzelt, 1987). Therefore, the Ca^{++}-uptake/sequestering activity of mitotic spindle ER membranes is very likely required for spindle MT integrity. Moreover, the localized release of Ca^{++} for example in the region of the kMTs, could specifically trigger the depolymerization of these MTs thereby inducing chromosome-to-pole motility.

In *Drosophila* syncytial embryos, unlike sea urchins, the ER is excluded from the mitotic spindle proper but instead forms the peripheral spindle envelope described earlier (Parry *et al.*, 2005, 2006) (Figure. 3(ii)). This envelope is proposed to function as a barrier between the spindle and surrounding cytoplasm that could facilitate the localized regulation of spindle Ca^{++} levels independent of bulk intracellular Ca^{++} concentrations, allowing

spindle-specific Ca^{++} signals to occur (Parry *et al.*, 2005). For example, the inositol trisphosphate (InsP3)-induced release of Ca^{++} from these membranes is proposed to trigger anaphase A chromosome separation but not anaphase B spindle elongation, based on experiments using InsP3 chelators/inhibitors (Parry *et al.*, 2005). Interestingly, extracellular Ca^{++} is also required for mitosis in this system, leading to the proposal that ER tubules might conduct the requisite Ca^{++} signals from the plasma membrane to the spindle (Parry *et al.*, 2006).

Similar mitotic spindle–associated Ca^{++} oscillations have also been observed in cells as diverse as land plant stamen hair cells and mammalian cultured cells (Ratan *et al.*, 1986; Hepler and Callahan, 1987), although in the latter case, the significance of the oscillations is debated (Tombes and Borisy, 1989; Nugues *et al.*, 2022). As in echinoderm embryos, manipulation of the Ca^{++} concentration within mammalian cell spindles can impact mitosis. For example, the experimental elevation of Ca^{++} during metaphase induces anaphase-like chromosome motility (Izant, 1983). Moreover, there is evidence that these cells contain ER membranes that become concentrated around the spindle poles by MT-based transport following NEB (Hepler and Wolniak, 1984; Waterman-Storer *et al.*, 1993) and a recent study revealed that an increase in the Ca^{++} concentration in the vicinity of these spindle pole membranes is essential for mitosis (Helassa *et al.*, 2019). It is possible that this could serve to depolymerize kMTs at the poles, thereby contributing to poleward MT flux and the flux component of anaphase A (Mitchison, 1989; Inoué and Salmon, 1995; Rogers *et al.*, 2004). Testing this and competing hypotheses about the role of Ca^{++} release from membranes localized around spindle poles requires further work.

Collectively these studies support the hypothesis that mitotic spindle–associated ER membranes control Ca^{++} concentrations within the spindle and regulate aspects of mitosis. It is plausible to think that highly localized Ca^{++} signals may act via Ca^{++}-regulatory proteins and downstream effector molecules to regulate specific mitotic events during specific phases of mitosis for example chromosome-to-pole motility during anaphase. However, much further work is required to bring us to a level of understanding comparable to that of the Ca^{++} regulation of muscle contraction. Specifically, it will be necessary to better define the exact sites of Ca^{++} release, the relevant Ca^{++}-binding targets and their downstream effectors for example MTs, motors and SAFs, within the mitotic spindle.

(iv): Membranes and membrane-derived protein assemblies as mechanical components of the spindle

In addition to controlling their chemical composition, membranes play important roles as mechanical components of mitotic spindles. For example, Hepler and Wolniak (1984) hypothesized that spindle membranes anchored at the poles might pull kMTs poleward, or that membranes within half spindles may function as substrates for MTs that glide along them to transport chromosomes poleward. Also, as in a currently outdated feature of the "replicon model" of Jacob *et al.* (1963) for bacteria, chromosome separation could be mediated by membrane growth and expansion between sisters. There now exists significant evidence supporting a role for spindle membranes and membrane-derived components in controlling the morphology, length and function of the spindle.

For example, the interesting review on mitosis in nonmodel systems by Drechsler and McAinsh (2012) describes the role and possible evolutionary implications of NM-bound microtubule organizing center (MTOCs) (e.g., in yeasts) and NE-bound kinetochores (e.g., in dinoflagellates). The mechanical coupling between the NE and the mitotic spindle observed during the closed mitosis of *S. pombe* suggests a reciprocal relationship in which not only does the elongating spindle contribute to nuclear division, but also the NE controls spindle function (Begley *et al.*, 2024). In the open and semi-open mitoses of human cells and *Drosophila* embryos, the prophase NE appears to function as a substrate for dynein-dependent centrosome separation in a process capable of complementing cortical dynein-driven and kinesin-5–driven spindle bipolarization (Robinson *et al.*, 1999; Sharp *et al.*, 2000; Raaijmakers *et al.*, 2012).

The role of the NE-derived NL in *Drosophila* embryo spindle mechanics was investigated using functional perturbations of mitotic motors in the presence of a GFP-lamin B reporter (Civelekoglu-Scholey *et al.*, 2010). This work suggested that a disassembling lamin B spindle envelope controls spindle length and morphology by stabilizing a mitotic motor–dependent force-balance, thereby buffering spindle length fluctuations (Figure 3(ii)). Following complete disassembly of the lamin B envelope by the end of prometaphase, spindle stability may be conferred by the two-layered, membranous metaphase spindle envelope (Stafstrom and Stahelin, 1984) and by the midzonal MT bundling Ase1 homologue, Feo during anaphase B (Wang *et al.*, 2015). Studies done in S2 cells suggest that the Rab5 GTPase, which regulates endosome trafficking and function during interphase, may contribute to the control of the dynamics and function of the nuclear lamin spindle envelope (Capalbo *et al.*, 2011). Rab5 associates with vesicles that locate around spindle poles as well as with nuclear lamins and the inhibition of its function using RNAi interferes with NL disassembly, proper chromosome congression and the timing of anaphase onset.

The hypothesis that nuclear lamin proteins derived from the disassembled NE contribute to spindle mechanics was extended to open mitoses in pioneering studies by Yixian Zheng and colleagues, who proposed that lamin B can assemble into a Ran.GTP-dependent "membranous spindle matrix" (Tsai *et al.*, 2006; Goodman *et al.*, 2010; reviewed in Zheng, 2010) (Figure 3(iii)). For example, in *Xenopus* egg extracts, at the onset of mitosis, disassembled lamin B is transported to the spindle poles during prometaphase and surrounds the spindle during metaphase, being more concentrated around the poles. This lamin B matrix, which maintains its spindle shape following MT disassembly (as do mitotic spindle membranes, e.g., Wright *et al.*, 1991), is proposed to facilitate spindle pole focusing and to maintain spindle length (Tsai *et al.*, 2006).

Further work by Zheng's group provided a link between the proposed lamin B spindle matrix and the process of liquid-liquid phase separation (LLPS) which has emerged as an important principle of intracellular organization (Hyman *et al.*, 2014). Briefly, LLPS describes the hydrophobic bond–driven formation of nonmembrane-bounded subcellular compartments (aka droplets or condensates) such as stress granules and nucleoli that facilitate biochemical pathways by concentrating and spatially organizing their components. In a sense, these compartments resemble membrane-associated biochemical compartments for example the mitochondrial electron transport chain (Labbé *et al.*, 2014; Mishra and Chan, 2014) but with the additional advantage that LLPS-driven condensates are transient and can form and disperse rapidly to perform their function in the cytoplasm, as is required during mitosis.

During their studies of the lamin B matrix in *Xenopus* egg extracts, Zheng's group focused on the low sequence complexity protein BuGZ, which acts upstream of lamin B during spindle

assembly (Jiang *et al.*, 2015). They observed that, in vitro, BuGZ can undergo a MT-independent, hydrophobic bond–dependent LLPS to form micron-scale liquid droplets capable of concentrating tubulin to facilitate MT assembly. [These droplets resemble similar spherical structures subsequently found in vivo (Grindheim *et al.*, 2024) as discussed later (Section (v)]. Moreover, they observed that inhibition of BuGZ function inhibits spindle assembly in frog extracts and mammalian cells. Thus, they hypothesize that the function of the lamin B spindle matrix involves the assembly of BuGZ condensates capable of concentrating tubulin and SAFs to facilitate spindle assembly (Jiang *et al.*, 2015). One such SAF is the aurora A mitotic kinase which is locally activated by phase separated BuGZ droplets to promote tubulin concentration and spindle assembly (Huang *et al.*, 2018). Such studies fuel speculation that many mitotic microtubule-associated proteins undergo LLPS and that this enhances their ability to control MT dynamics and spindle morphogenesis, a suggestion that merits further research (Sun *et al.*, 2024).

Returning to a discussion of the spindle envelope, Araújo *et al.* (2022) have now obtained evidence that ER membranes are required for normal spindle mechanics throughout mitosis in *Drosophila* syncytial embryos. They microinjected an inhibitor of ER-ER membrane fusion which disrupted ER organization, especially around the spindle poles, and observed a decrease in spindle length and a reduced rate of poleward chromosome motility, resulting in defects in nuclear spacing following mitotic exit. The authors conclude that ER membranes concentrated around the spindle poles act as mechanical elements that serve to complement forces generated by spindle MTs and mitotic motors. Thus, they are continuously required to maintain the spindle force-balance responsible for normal spindle size, rate of chromosome motility, and nuclear spacing.

A somewhat similar ER reticulum, the "centriculum," which forms in a centrosome- and MT-dependent manner and surrounds the mitotic spindle poles of one-cell *C. elegans* embryos, is also thought to contribute to spindle function (Maheshwari *et al.*, 2023). This centrosome-associated centriculum fuses with the NE at metaphase and is proposed to control spindle MT number and orientation by; (i) restricting the size of the centrosome to limit the number of available MT nucleation sites; and (ii) sterically blocking the growth of suboptimally directed elongating MTs. In addition, it's mechanical connection to the NE allows it to assist in the process of NE fenestration and breakdown as the spindle poles are pulled apart.

Other endomembrane-derived spindle membranes appear to contribute to spindle mechanics by serving as MTOCs that promote MT assembly and mitotic spindle organization. For example, the Golgi apparatus, which contributes to MT dynamics and organization in interphase vertebrate cells (Sanders and Kaverina, 2015), yields vesicles that associate with aMTs and concentrate around the spindle poles (Figure 2, A and B). These Golgi vesicles are proposed to stimulate MT assembly and spindle attachment via a pathway involving the peripheral Golgi matrix protein, GM130, as well as importin, TPX2 and Aurora A kinase. Consequently, the antibody-mediated inhibition of GM130 function led to impaired spindle formation, mitotic arrest and the inhibition of cell division (Wei *et al.*, 2015). Endosome-associated Rab GTPases are known to be required for normal aster size, spindle morphology, and cell division in *C. elegans* embryos (Skop *et al.*, 2001). Such Rab proteins also associate with endosomal vesicles concentrated at the spindle poles of Hela and *Drosophila* cells in a dynein-dependent manner, where they appear to recruit SAFs for example NuMA, pericentrin, and EB1, and regulate the nucleation of MT assembly and mitotic spindle function (Capalbo *et al.*, 2011; Hehnly and Doxsey, 2014).

In addition, the vesicle coat protein, clathrin, which functions in clathrin-mediated endocytosis during interphase (Figure 1), localizes to the mitotic spindle in cultured mammalian and tobacco cells, and its depletion leads to mitotic defects including abnormal chromosome congression (Royle *et al.*, 2005; Tahara *et al.*, 2007). However, mammalian clathrin appears to carry out its mitotic functions in a membrane-independent manner, notably by cooperating with TACC3 and chTOG proteins to cross-link kMTs into bundled kinetochore fibers. Thus, clathrin is proposed to be one of several membrane trafficking proteins that are derived from intracellular membranes but "moonlight" as mitotic spindle proteins in a membrane association-independent manner (Royle, 2013).

(v): Active membrane partitioning mediated by the mitotic spindle

While membranes contribute to mitotic spindle composition and function, conversely it is plausible to hypothesize that the spindle may contribute to the segregation, inheritance, and functional organization of intracellular membranes. It is understood that, like chromatin, membranous organelles undergo dramatic reorganization for proper distribution to the daughter cells during cell division. However, much remains unknown about this partitioning process and researching the relative roles of active segregation of mitotic organelles mediated, for example, by the spindle cytoskeleton versus passive, stochastic organelle partitioning is an active field.

The long-standing, stochastic inheritance theory proposes that relatively large organelles such as the Golgi fragment to produce multiple small vesicles that disperse randomly throughout the cytoplasm in preparation for mitosis. In this model, these vesicles, together with smaller organelles such as individual mitochondria and components of the endosome-lysosome network, are partitioned passively and stochastically during cell division, followed by their reassembly for the subsequent interphase (Carlton *et al.*, 2020). However, recent work suggests that some of these membranous structures may also be actively partitioned Figure 2, A and B). For example, Golgi-derived vesicles and endosomes cluster at the poles of the mitotic spindle in some cells, which may aid in their active segregation, plausibly by pole-pole separation during spindle elongation. Moreover, the Golgi may merge with the ER/NE network in preparation for mitosis, forming a continuum that remains intact rather than fragmenting into vesicles, and which can be actively partitioned by the spindle (Figure 2B) (Terasaki, 2000; Carlton *et al.*, 2020; Kors and Schlaitz, 2024).

For instance, the mitotic spindle contributes to the active, cell cycle–regulated partitioning of the ER in *Drosophila* syncytial embryos, meiotic spermatocytes and neuroblasts, where the ER concentrates in, and partitions with, the asters (consisting of radial arrays of aMTs emanating from a centrosome) (Bergman *et al.*, 2015; Smyth *et al.*, 2015; Diaz *et al.*, 2019). In these cells, disruption of the asters led to abnormal ER partitioning, suggesting that the active segregation of the asters is required for correct ER inheritance in both symmetric and asymmetric mitoses. Moreover, observations of the asymmetric partitioning of the ER associated with the different-sized asters of asymmetrically dividing neuroblast mitotic spindles provides strong evidence that aster-associated ER membranes are actively segregated (Smyth *et al.*, 2015). It is plausible to think that the extensive network of ER membranes, including the absorbed Golgi membranes, that associate with sea urchin

embryonic spindles is similarly actively partitioned (Henson *et al.*, 1989; Terasaki and Jaffe, 1991; Terasaki, 2000).

The mitotic spindle plays an active role in NE partitioning during the closed mitosis of the fission yeast *S. pombe*. Here, motor-driven elongation of the spindle drives the formation of daughter nuclei via a dumbbell-shaped intermediate connected by a narrow bridge where localized NEB and NPC disassembly occur prior to cell division (Dey and Baum 2021; Gergely *et al.*, 2023; Krüger *et al.*, 2021) (Figure 3(i)). Mechanical coupling of the elongating spindle to the NE via the spindle pole bodies, together with a broadening of the area of force generation by separated chromosomes localized at the poles, are thought to facilitate the NE shape changes associated with nuclear division (Zheng *et al.*, 2007; Zhu *et al.*, 2016). Moreover, when mitotic spindle function is impaired, nuclei can only divide by an inefficient "nuclear fission" mechanism (Castagnetti *et al.*, 2010).

In some mammalian cells, it appears that spindle disassembly may play an important role in NE partitioning (Vietri *et al.*, 2015; Kelley *et al.*, 2024). Here, spindle MTs can hinder the reassembly of the NE in daughter cells by sterically blocking the association of the NE with late anaphase chromosomes. To prevent this, the MT severing ATPase, spastin normally catalyzes the removal of such interfering MTs. However, under conditions in which this MT-severing mechanism is impaired, the dividing cell can compensate by creating NL-lined membranous "micro tunnels" that enclose any abnormally persistent MTs and allow reformation of the NE irrespective of the presence of excess MTs.

Subpopulations of components of the secretory and endocytic endomembrane system can be actively partitioned by the mitotic spindle as well (Figures 1 and 2). With respect to the exocytic pathway of mammalian cells, for instance, Golgi vesicles associate with mitotic spindle MTs around the poles with the aid of the Golgi matrix protein, GM130, and this association is likely to facilitate their active partitioning (Shima *et al.*, 1998; Wei *et al.*, 2015; Ayala and Colanzi, 2022). Intermediate compartment (IC) membranes also accumulate at the spindle poles where they are proposed to contribute to Golgi partitioning/reassembly (Marie *et al.*, 2012). Exocytotic vesicles that emerge from the spindle-associated ER/Golgi network and are delivered by kinesin-1 to the cell surface in sea urchin blastomeres represent another example (Wright *et al.*, 1991; Bi *et al.*, 1997). Examples of endolysosomal organelles that are likely to be actively partitioned by the mitotic spindle include lysosomes that dock around prometaphase and metaphase chromosomes and telophase spindle poles (Hämälistö *et al.*, 2021) and Rab 11 endosomes which become clustered around the spindle poles by dynein-driven retrograde transport along aMTs (Hehnly and Doxsey, 2014). Interestingly, Grindheim *et al.*, 2024 recently described the formation in prometaphase cultured rat cells of aMT-associated lamin B/annexin A2-bound spherical structures which specifically contain IC and recycling endosome components. Analysis of late anaphase cells suggests that equal numbers of these particles are delivered to the daughters, suggesting that these structures mediate the selective, spindle-driven partitioning of the IC and recycling endosomes (Grindheim *et al.*, 2024). The role of spindle MTs in this process requires further analysis, however.

Mitochondria provide an interesting case to consider because they appear to undergo a situation-dependent combination of both active and passive partitioning. These critical, energy-generating organelles are known to undergo dramatic morphological changes that depend upon dynamin GTPase-regulated episodes of fusion and fission (Labbé *et al.*, 2014). Thus, in mammalian cells, they fuse into large networks during interphase, but then fragment by fission during mitotic entry which is widely believed to permit their passive, stochastic inheritance during cell division (Carlton *et al.*, 2020). However, stochastic inheritance is unlikely to be the only method of mitochondrial segregation because active, actin-based mitochondrial partitioning by myosin-V occurs in budding *S. cerevisiae* cells, and the attachment of mitochondria to aMTs in mammalian cells may underlie an active, spindle-based segregation mechanism (Mishra and Chan, 2014; Lawrence *et al.*, 2016; Carlton *et al.*, 2020). This is probably the case in human cells where the motor adaptor, Miro and the kinetochore protein, Cenp-F facilitate active, MT tip tracking-dependent mitochondrial partitioning during late mitosis (Kanfer *et al.*, 2015). During early mitosis, however, mitotic kinases mediate the dissociation of motors from mitochondrial membranes, which is necessary for; (i) mitochondrial detachment from spindle MTs; (ii) passive, symmetrical mitochondrial inheritance; and (iii) normal mitosis (Chung *et al.*, 2016). Possibly the release of mitochondria from spindle MTs is not only required to enable their stochastic partitioning but also to allow them to cluster into layers surrounding the spindle periphery (Hepler and Wolniak, 1984) (Figure 2). It would be interesting to know whether the formation of such layered mitochondrial arrays facilitates the delivery by diffusion of enough ATP to supply mitotic ATPases for example motors and kinases, and to what extent other sources of ATP, for example, glycolysis and creatine kinase, contribute to fueling the spindle. In this context, it was recently proposed that an increase in the formation of membrane contact sites between the ER and these mitochondria during mitosis promotes mitochondrial Ca^{++} influx, which in turn up-regulates ATP synthesis to satisfy the expanded energetic demands of mitosis and cell division (Zhao *et al.*, 2024). Finally, a recent study of the unusual "one mitochondrion per cell" inheritance mechanism of the malaria-causing protist, *Plasmodium*, in which the mitochondrion orients into a "cartwheel" intermediate structure prior to cell division, underscores the diversity of partitioning mechanisms, both active and passive, that may exist for this critical organelle (Verhoef *et al.*, 2024).

(vi): The Spindle as a reservoir of partitioned membranes for delivery to the cytoplasm, plasma membrane, extracellular matrix, and cleavage furrow

Membranous organelles that have been actively partitioned by the mitotic spindle may be redistributed to the cytoplasm of daughter cells at the end of M-phase to help reform the interphase array and serve as a reservoir of membrane for delivery to the cell membrane and cleavage furrow (Figure 2).

For example, the anterograde organelle transport motor, kinesin-1 (Vale *et al.*, 1985), colocalizes with ER membranes that concentrate in early sea urchin embryo mitotic spindles (Wright *et al.*, 1991). Inhibiting kinesin-1 activity by antibody microinjection did not perturb mitosis, cell division or spindle-associated ER organization in these cells, but it did inhibit the delivery of exocytic vesicles to the cell surface (Wright *et al.*, 1993; Bi *et al.*, 1997) (Figure 2B). Specifically, wounding the plasma membrane to trigger vesicle recruitment for Ca^{++} regulated exocytosis-dependent repair was found to require long-range transport of exocytic vesicles along aMTs by kinesin-1, followed by short-range transport along cortical actin by a BDM-sensitive myosin (Bi *et al.*, 1997; Forer and Fabian, 2005). After partitioning by the spindle (Figure 2B), the radial dispersion of these stockpiled ER-Golgi membranes into the cytoplasm may help generate the endomembrane network of blastomeres as they form in the developing embryo (Wright *et al.*, 1991). Furthermore, the transport of exocytic vesicles derived

from these membrane stores could contribute to other physiological processes for example extracellular matrix assembly (Matese *et al.*, 1997). Similarly, heterotrimeric kinesin-2–bound vesicles that accumulate in sea urchin mitotic spindles (Henson *et al.*, 1995), may be actively partitioned to the forming blastomeres throughout early embryogenesis, then delivered to the apical surface of blastula-stage cells when needed to support ciliogenesis (Morris and Scholey, 1997). This type of mechanism may also explain the spindle localization of the IFT motor, kinesin-2 in *Chlamydomonas* (Vashishtha *et al.*, 1996).

Anterograde transport of membranes stockpiled in and partitioned by the spindle is also important for the plasma membrane rearrangements that accompany animal cell cytokinesis, which occurs in three steps, namely, (i) determination of the cleavage planes; (ii) the assembly, constriction and disassembly of actomyosin-based contractile ring; and (iii) midbody formation and abscission (Pollard and O'Shaughnessy, 2019). In some cells, the unfolding of accumulated, folded plasma membrane reservoirs is sufficient to support the shape changes accompanying furrow ingression (Alonso-Matilla *et al.*, 2024). However, ingression often requires the delivery of new membrane to the furrow region by motor-dependent trafficking of spindle-associated exocytic and endocytic vesicles (Vallee *et al.*, 1990; Albertson *et al.*, 2005; McKay and Burgess, 2011). Accordingly, both exocytic and endocytic vesicles accumulate on the late spindle interzone/midbody of mammalian cells and deliver membrane to the cleavage furrow in a process controlled by cell cycle kinases (Figure 2B) (Goss and Toomre, 2008; Schweitzer *et al.*, 2005; Brose *et al.*, 2017). Vesicle motors associated with both MTs for example kinesin-1 and dynein/dynactin (Montagnac *et al.*, 2009) and actin for example the minus-end–directed myosin-VI (Arden *et al.*, 2007), are proposed to play essential roles in targeted membrane delivery for cytokinesis.

In sea urchin blastomeres, an increase in plasma membrane surface area during cytokinesis is proposed to involve exocytic vesicle delivery to two sites. First to the poles of the cell following transport along aMTs and cortical actin during anaphase (Gudejko *et al.*, 2012) and later to the equator in a process requiring overlapping aMTs during telophase (Shuster and Burgess, 2002) (Figure 2B). Kinesin-1 localizes to ER membranes throughout the half spindles and asters whereas kinesin-2–associated vesicles localize differently, throughout the metaphase half spindles and anaphase central spindle (Wright *et al.*, 1991; Henson *et al.*, 1995). Given the evidence that both these motors are required for the completion of cytokinesis in vertebrate cells (next paragraph), it is tempting to speculate that kinesin-1 and -2 could deliver vesicles to the poles and equator of the cell, respectively. However, mAb inhibition of neither kinesin-1 or-2 interfered with cytokinesis so either this hypothesis is wrong, functional redundancy between these motors obscures their role in cytokinesis, or different motors are responsible. Now that the cytoskeletal motility proteins encoded by the sea urchin genome have been catalogued, perhaps a systematic attack on this problem is possible (Morris *et al.*, 2006).

The final stage of cytokinesis involves the disassembly of the actomyosin contractile ring and the formation of the midbody, an antiparallel array of MTs with associated vesicles and motors, encircled by the "midbody ring" at its center, which forms an intercellular bridge between the two daughter cells. The midbody resembles the phragmoplast of land plants (Euteneuer and McIntosh, 1980; Skop *et al.*, 2001; Lee and Liu, 2013) and it coordinates abscission, the physical separation of the plasma membrane of the two daughter cells (Barr and Gruneberg, 2007). This is a poorly understood process that requires the delivery of spindle-associated exocytic and endocytic vesicles to the midzone and midbody ring (Figure 2B) similar to motor-driven transport observed in the phragmoplast (Yamada et al., 2025). In mammalian cells, kinesin-1 is proposed to cooperate with dynein/dynactin to traffic recycling endosomes along the intercellular bridge; kinesin-2 is reported to move exocytic vesicles on the spindle midzone/midbody and endosomes from the spindle poles via the midzone to the cleavage furrow; and myosin-VI transports vesicles that concentrate around the spindle poles to the midbody and furrow, thereby contributing to abscission (Montagnac *et al.*, 2009; Fan and Beck, 2004; Arden *et al.*, 2007; Li *et al.*, 2014; Pupo *et al.*, 2018). In the final stage of abscission, the intercellular bridge is cleaved on either side of the central ring which breaks the last remaining connection between the daughter cells, thereby releasing the central midbody remnant whose degradation may involve the action of spindle-associated lysosomes or autophagosomes (Barr and Gruneberg, 2007; Hämälistö *et al.*, 2021).

SUMMARY AND CONCLUSIONS

Mitotic spindle membranes were not a major focus of my laboratory's research, but our chance finding that kinesins-1 and -2 colocalize with membranous structures in sea urchin embryo spindles (Wright *et al.*, 1991; Henson *et al.*, 1995) motivated me to follow work on this complex topic with great interest over the years. From my perspective, the study of spindle membranes seemed to take a back seat in the field of mitosis research following the publication 40 years ago of the classic review by Hepler and Wolniak (1984). This is possibly because of the field's justifiable excitement and focus on the discovery of the precise three-dimensional organization of MTs in several mitotic spindles, the discovery of MT dynamic instability and poleward flux, and the recognition of the importance of centrosomes, chromosomes, and MT-based mitotic motors for the mechanism of mitosis. In my opinion, the possibility that some membranous structures may associate with spindles in a nonphysiological manner has not been eliminated (Wadsworth, 1987). However, the study of spindle membranes has clearly attracted renewed interest during the past couple of decades and several excellent papers that underscore the biological significance of spindle membranes have appeared, as I have attempted to summarize here. Notable progress has been made in improving understanding of the contribution of membranous components to spindle composition, mechanics and dynamics and conversely on the role of the mitotic spindle in the partitioning, dynamics and redistribution of its membrane components. Many questions remain to be answered and future work on this exciting topic should further clarify the significance and functions of membrane–spindle interactions and will perhaps yield unexpected surprises.

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