Recent Glimpses of the Elusive Spindle Matrix

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ABSTRACT

A stationary spindle matrix has been proposed on theoretical grounds to help mediate force production at the mitotic spindle. Direct molecular evidence for the existence of such a matrix has the potential to profoundly influence our view of the molecular mechanisms leading to chromosome segregation during mitosis. Three recent papers suggest that the spindle matrix may be more than a theoretical idea.

INTRODUCTION

A basic feature of cell division is the formation of a mitotic spindle that helps segregate each set of the duplicated chromosomes to the daughter nuclei. The spindle apparatus is a complex molecular machine known to be made up of polymerized tubulin and various associated motor proteins. While numerous models have been proposed for how the spindle apparatus may transmit forces none of these models have been completely satisfying. One of the main problems is the inherent dynamic instability of microtubules themselves. The discovery of microtubule flux at both ends of spindle microtubules and of the constant treadmilling of tubulin dimers toward the poles makes it difficult to envision how forces are generated to actually move the chromosomes on the basis of a metastable structure that is not anchored in place. For these reasons and based on theoretical considerations of the requirement for force production at the spindle the concept of a spindle matrix has long been proposed. In its simplest formulation a spindle matrix is hypothesized to provide a more or less stationary substrate that provides a backbone or strut for motor molecules to interact with during force generation and microtubule sliding. Such a matrix could also be envisioned to have the added properties of helping to organize and stabilize the microtubule spindle. Although there have been some tantalizing hints in the literature for the actual existence of such a spindle matrix direct molecular or biochemical evidence has been elusive. However, the results from three recent studies reviewed here breathe new life into the spindle matrix concept and promises to bring us much closer to verifying its physical reality.

SKELETOR, THE FOUNDING MEMBER OF A COMPLEX OF SPINDLE MATRIX PROTEINS IN DROSOPHILA

Skeletor is an 81 kD protein that was identified by use of a mAb with an intriguing dynamic staining pattern during mitosis in Drosophila embryos. Skeletor antibody labeling shows that Skeletor is associated with chromosomes at interphase but redistributes into a true fusiform spindle at prophase which precedes microtubule spindle formation (Fig. 1). During metaphase the "Skeletor-spindle" and the microtubule spindles are co-aligned (Fig. 1). Importantly, the Skeletor-defined spindle maintains its fusiform spindle structure from end to end across the metaphase plate during anaphase when the chromosomes segregate (Fig. 1). At telophase the chromosomes start to decondense and reassociate with Skeletor where the two daughter nuclei are forming while Skeletor still defines a spindle in the midregion (Fig. 1). Thus, the Skeletor-defined spindle exhibits all the properties predicted for the spindle matrix. Especially, the finding that the Skeletor-defined spindle matrix maintains its fusiform spindle structure during chromosome segregation makes it an ideal candidate for being a scaffold that provides structural support for motor proteins and counterbalancing force production. That the Skeletor spindle may provide a substrate for the alignment of microtubules is further supported by the finding that microtubules are co-localized with the spindle matrix that remains in the central region during...
midbody formation at telophase. This alignment of microtubules at the midbody region is largely unaccounted for by most other models but could be explained if the Skeletor spindle as hypothesized by Walker et al. helps in organizing microtubule fibers. However, Skeletor encodes a low-complexity protein with no obvious motifs making it unlikely that Skeletor itself is a structural component of the spindle matrix but rather that it is a member of a multi-protein complex. Consequentially, in order to identify other members of such a complex Wang et al. performed yeast two-hybrid interaction assays as well as coimmunoprecipitation analysis. In preliminary experiments using these approaches a novel chromdomain containing protein, which was named Chromator, was found to interact with Skeletor. A mAb generated against Chromator showed that it has a dynamic cell cycle specific pattern similar but not identical to that of Skeletor. A third protein that in immunolabelings completely co-localizes with Skeletor during mitosis was also identified. This protein displays coiled-coil motifs suggesting it may comprise a structural component of the spindle matrix. Analysis of a P-element insertion into this gene suggests that a reduction in protein levels encoded by this locus results in spindle abnormalities and chromosome segregation defects and that the protein is essential for viability. Thus, these findings indicate that Skeletor is not alone but may be the founding member of an interacting complex of nuclear components that act in concert to form a spindle matrix. The identification of several of these other players and the availability of P-element insertions in some of their genes promises to pave the way for a direct structural as well as functional analysis of the spindle matrix in *Drosophila*.

**THE KINESIN EG5 AND MICROTUBULE FLUX IN XENOPUS EXTRACT SPINDLES**

Using a very different approach Kapoor and Mitchison studied the distribution and dynamics of the motor protein Eg5 in *Xenopus* spindles relative to that of the microtubules. Eg5 previously had been shown to localize throughout the spindle and play a key role in establishing its bipolar organization. Kapoor and Mitchison applied the recently developed “fluorescence speckle microscopy” (FSM) technique that relies on random variation of incorporation of normal and fluorescently-tagged subunits within a macromolecular assembly (such as a microtubule) to create a “speckled” pattern. These speckles can then provide fiduciary marks serving as reference points to follow movement or flux of the structure over time using simple and non-perturbing CCD camera imaging approaches. By applying FSM to compare the mobility of fluorescently speckled microtubules with that of the microtubule-binding motor protein Eg5 Kapoor and Mitchison made the surprising discovery that the vast majority of Eg5 in the spindle is static despite the continuous poleward flux observed for microtubules. A number of important controls showed that the recombinant, fluorescently-tagged Eg5 behaved similarly to the endogenous Eg5:

1. in biophysical assays the recombinant protein displayed the proper Stokes radius,
2. it promoted microtubule gliding with the same velocities,
3. its localization to the spindle was indistinguishable from that of wild-type, and
4. it was capable of rescuing bipolar spindle formation from Eg5-depleted extracts.

Furthermore, the fact that excess Eg5 added to control experiments did not alter spindle assembly or microtubule dynamics in any measurable way suggests that the assays were not being influenced by variations in Eg5 protein level. Thus, this raises the conundrum of how a microtubule-binding motor protein could remain fixed in place on a constantly treadmilling spindle. One possible explanation would be if the motor protein was “walking” on the microtubule in the opposite direction to its poleward flux at an equal but opposite rate of speed. However, this explanation is unlikely since when monastrol, a specific inhibitor of Eg5 motility, was included in the assays similar results were observed. Had the motor been counter-acting the poleward microtubule flux, the Eg5 should have been carried poleward with the microtubules once its motor was inactivated. Also unlikely is that Eg5 itself associates with a class of stationary microtubules as there was no evidence from the FSM measurements of a proportionate number of static microtubules. Thus, the authors suggest the immobility of Eg5 on mitotic spindles may be due to an interaction with a static spindle matrix.

Interestingly, the static behavior observed for Eg5 in the above studies appears inconsistent with results reported by Wilde et al. who used fluorescently-tagged antibodies against Eg5 to speckle-label asters emanating from centrosomes added to cytostatic factor-arrested *Xenopus* egg extracts in the presence of Ran-GTP. In these studies the Eg5-speckles moved towards the plus end of micro-

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**Figure 1.** The dynamics of Skeletor spindle matrix formation and distribution during mitosis as compared to the microtubule spindle. Confocal images were obtained from syncytial *Drosophila* blastoderm embryos labeled with mAb 1A1 for Skeletor (red), with anti-tubulin antibody (green), and with Hoechst for DNA (blue). The composite images of the labelings (comp) are shown to the left. The labelings show that in late prophase, when the microtubules have not yet entered the nuclear space, the Skeletor spindle is already aligned within the nucleus. During metaphase the two spindles are co-extensive, although the Skeletor spindle appears broader than the microtubule spindle. At anaphase the Skeletor spindle persists as an intact spindle extending across the metaphase plate as the chromosomes segregate to the poles. During telophase the chromosomes have segregated and as they decondense, they reassociate with Skeletor at the poles. However, the Skeletor spindle persists in the central region where midbody formation of the microtubules is found to take place in alignment with the remaining Skeletor spindle structure.
FIN1P FORMS NON-MICROTUBE FILAMENT IN YEAST NUCLEI

A third glimpse of a potential spindle matrix protein came from studies in yeast. In a two-hybrid interaction screen to identify binding partners of the S. cerevisiae Fin1p, that forms a filamentous structure extending between the two nuclei of dividing cells. Intriguingly, this structure was observed to colocalize with the microtubules at this stage. Furthermore, the distribution and organization of Fin1p changes dynamically during the cell cycle as Fin1p in non-dividing cells is nuclear but not filamentous and distinct from the microtubules. The ability to form filaments appears inherent to the Fin1 protein since 6xHis-tagged Fin1p isolated from yeast extract could self-assemble into 10 nm filamentous structures independently of microtubules or other proteins in vitro. The finding of a coiled-coil protein that can form filaments independently of microtubules yet co-localizes with the microtubules during mitosis hints towards a potential presence of a stationary scaffold in the yeast mitotic spindle as well. The significance or role of this scaffold in yeast is not yet clear as current studies suggest that yeast spindle microtubules do not “treadmill” but rather assemble and disassemble from their plus ends. Nonetheless, the majority of microtubules in yeast metaphase spindles are still highly dynamic and curiously, during anaphase, the entire spindle reorients itself relative to the mother-bud neck while simultaneously elongating. Thus, in yeast questions pertain not only to how force production is inherent to the Fin1 protein since 6xHis-tagged Fin1p isolated from yeast extract could self-assemble into 10 nm filamentous structures independently of microtubules or other proteins in vitro. The finding of a coiled-coil protein that can form filaments independently of microtubules yet co-localizes with the microtubules during mitosis hints towards a potential presence of a stationary scaffold in the yeast mitotic spindle as well. The significance or role of this scaffold in yeast is not yet clear as current studies suggest that yeast spindle microtubules do not “treadmill” but rather assemble and disassemble from their plus ends. Nonetheless, the majority of microtubules in yeast metaphase spindles are still highly dynamic and curiously, during anaphase, the entire spindle reorients itself relative to the mother-bud neck while simultaneously elongating. Thus, in yeast questions pertain not only to how force production is generated but also to how mitotic apparatus repositioning can be effected on rapidly remodeling structures such as microtubules. Whether Fin1p may play a role in these processes will be of great interest to probe in future studies. The fact that the fin1 null mutant is viable may indicate redundancy and that there are other components of a potential yeast spindle matrix yet to be discovered.

CONCLUSION

Studies using preparations spanning the evolutionary spectrum from lower eukaryotes to vertebrates have provided new and intriguing evidence that a spindle matrix may be a general feature of mitosis. Especially, the identification of several potential spindle matrix molecules in Drosophila together with P-element mutations in their genes should provide an avenue for genetic and biochemical experiments directly testing the functional concept of the spindle matrix’s role in chromosome segregation and force production. These experiments have the potential to finally resolve whether the spindle matrix is a physical reality or a mirage.

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References