

The spindle matrix through the cell cycle in *Drosophila*

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A spindle matrix has long been proposed to provide structural support for counterbalancing force production and a substrate for essential mitotic factors. For years the molecular identity of such a structure remained elusive. Recently a complex of nuclear proteins that reorganize into a spindle-like structure during prophase through metaphase that shows characteristics of a spindle matrix has been identified in *Drosophila*. We review how these results support the concept of a spindle matrix and discuss its possible function(s) during mitosis. Importantly, these molecules also appear to play critical roles during interphase in nuclear organization and function. Given that during cell division the entire nucleus undergoes a dynamic and tightly orchestrated reorganization, the reorganization of spindle matrix components during mitosis may comprise one phase of a continuum of “nuclear architectural remodeling events” that can be considered to extend throughout the entire cell cycle, even in the absence of a defined nucleus.

Introduction

During cell division the entire nucleus undergoes a dramatic reorganization as the cell prepares to segregate its duplicated chromosomes. For many years the prevailing view in organisms possessing an open mitosis held that the nucleus disassembled during early mitotic stages, thus enabling cytoplasmic microtubules emanating from the separated centrosomes to form a mitotic spindle, until the end of telophase when two daughter nuclei reassembled around the decondensing segregated chromosomes.¹ This cytocentric view discounted any nuclear contributions to the formation and/or function of the mitotic spindle. Increasing evidence suggests, however, that nuclear contents do play critical roles in constructing and regulating the mitotic machinery. Chromosomes themselves can promote the assembly of microtubules that are focused to form a spindle as first observed for acentrosomal cells (reviewed in ref. 2) but now accepted as a general mechanism contributing to spindle assembly even in cells with centrosomes.^{3,4} *Drosophila* provided a particularly amenable model system to elaborate this so-called “self-organization” mode of spindle assembly due to the availability of mutants that compromised the centrosomal “search and capture” mode of spindle assembly.⁵⁻⁷ More recently additional nuclear contributions to spindle assembly have been revealed by

results in *Drosophila* that provided molecular evidence supporting the existence of a spindle matrix that has long been proposed based on theoretical grounds but whose composition until recently had been difficult to define (reviewed in ref. 8).

Long before the role of chromosomes in spindle organization became clear, evidence from a wide range of studies pointed towards the existence of a non-microtubule component of the mitotic spindle. Early studies revealed a large volume of non-microtubule components in the spindle,^{9,10} a finding supported by EM evidence and birefringence studies that suggested a non-microtubule component.¹⁰⁻¹² Different extraction protocols yielded a non-microtubule “spindle remnant” in the absence of microtubules.¹³⁻¹⁶ Indeed, the idea of a such non-microtubule matrix that would assist in spindle formation and/or function had earlier been proposed to account for properties of the spindle that were not consistent with a spindle comprised solely of microtubules and motors.¹⁷⁻¹⁹ For example, it is difficult to envision how motor proteins can generate the forces needed to move the chromosomes on a treadmill substrate such as spindle microtubules that themselves are constantly in flux.^{20,21} Furthermore the balance of plus-end-directed and minus-end-directed motors in the spindle results in a state of constant tension.²²⁻²⁵ With 1- to 10 pN of force generated per motor²⁶⁻²⁹ the combined force from the hundreds to thousands of motors in the spindle measures out to ~1,000 pN.³⁰ Such force would buckle microtubules *in vitro*³¹⁻³³ yet buckling does not occur in the spindle, suggesting that additional measures of support are present some of which might be provided by a spindle matrix. Especially compelling that spindle function involves more than just microtubules and motors, however, are experiments showing that poleward chromosome movement persists even after the kinetochore microtubules are severed using a UV microbeam.^{19,34-36} These results along with the observation that despite constant microtubule flux, mitotic spindles maintain a uniform size characteristic of that particular cell type³⁷ have sparked much interest in identifying spindle-associated factors to assess whether they may comprise part of a spindle matrix.

In considering whether mitotic spindle-associated molecules comprise a bona fide “spindle matrix”, it is important to consider the characteristics that would be expected for such a structure: (1) they should form a true fusiform structure coalescent with spindle microtubules; (2) they should persist in the absence of microtubules; (3) perturbation of one or more members of the complex should affect spindle assembly and/or function; (4) one or more components should interact with microtubules and/or microtubule-associated motor proteins.⁸ Such a matrix may provide scaffolding functions to localize required spindle checkpoint- and

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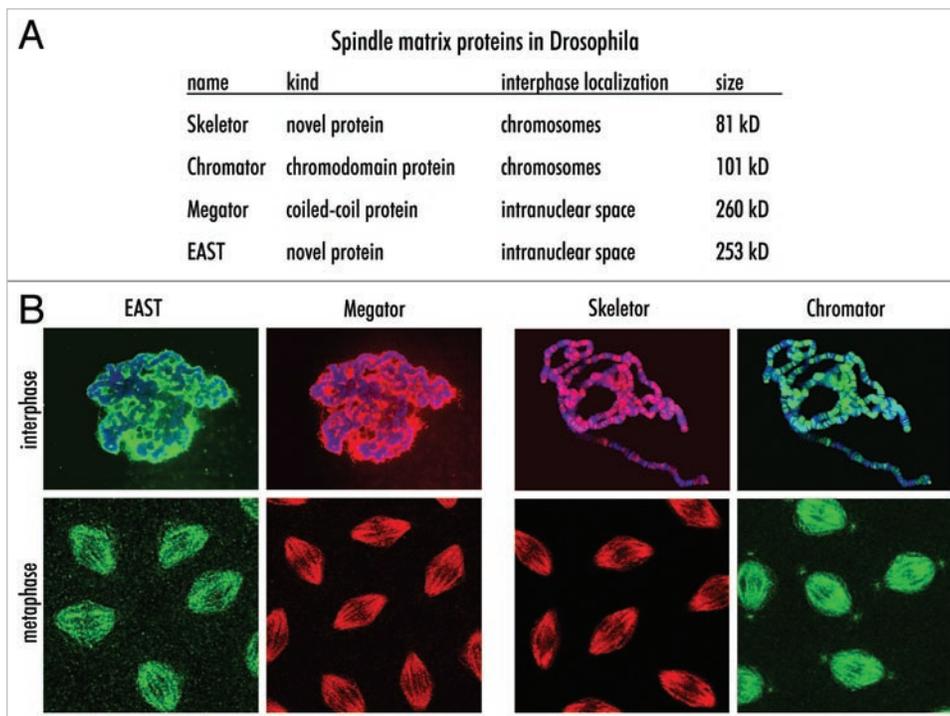


Figure 1. Spindle matrix proteins in *Drosophila*. (A) Characteristics of the four spindle matrix proteins Skeletor, Chromator, Megator and EAST. Skeletor and EAST have no identified structural motifs, Chromator contains a chromodomain, while Megator contains a long coiled-coil domain and is the human Tpr ortholog. (B) Localization of spindle matrix proteins. Upper: During interphase EAST (green) and Megator (red) localize to the interchromosomal domain, appearing to fill the space around the chromosomes (blue) as visualized in confocal images of light squashes of nuclei from polytene larval third instar salivary glands. In contrast, Skeletor (red) and Chromator (green) localize to interband regions of the chromosomes (blue) observable in standard polytene squashes from larval third instar salivary glands. Lower: All four proteins reorganize during mitosis to form a spindle-like structure at metaphase as shown in confocal images from dividing syncytial embryonic nuclei. In addition, Chromator localizes to the centrosomes. Spindle matrix proteins are detected with antibodies specific to each of the four spindle matrix proteins (red or green); DNA is stained with Hoechst (blue).

assembly-factors as well as contribute directly to force production within the spindle. Recently a complex of proteins exhibiting such properties has been identified in *Drosophila*³⁸⁻⁴¹ (**Fig. 1**) and further study of these proteins promises to shed light on the form and mechanistic function of the elusive spindle matrix.

Molecular Components of a Spindle Matrix in *Drosophila*

The first candidate spindle matrix protein in *Drosophila*, Skeletor³⁸ was identified due to a serendipitous cross-reactivity of the monoclonal antibody mAb2A that failed to recognize its intended target, Notch, but instead stained a meshwork-like structure at interphase that reorganized to a spindle-like structure at metaphase.⁴² What was particularly suggestive of a link to the spindle matrix was that the Skeletor-defined spindle structure begins to form during prophase (**Fig. 2**) at a time when tubulin is excluded from entering the nucleus and it persists even after the microtubules have been disassembled with nocodazole.³⁸ The *Skeletor* gene maps to 86C1-2 on the third chromosome and encodes a low complexity protein with no obvious structural motifs,³⁸ suggesting it likely interacts with other proteins that would function to

specify the architectural aspects of the spindle matrix. Thus, having isolated one such candidate spindle matrix protein, it was possible to identify other members of the complex by yeast two-hybrid screening and co-immunoprecipitation methods. Such methods have until now yielded four nuclear proteins that reorganize to associate with the spindle matrix during mitosis: Skeletor, Chromator, Megator and EAST (**Fig. 1**).³⁸⁻⁴¹

Chromator was originally identified as a two-hybrid interaction partner with Skeletor and contains an N-terminal chromodomain and both Skeletor- and EAST-interaction domains in its C-terminus.^{39,43} The *Chromator* gene maps to the third chromosome at 79F near the pericentromeric heterochromatin. The locus gives rise to at least three alternative transcripts with each encoding the same 926 amino acid protein. Although the predicted molecular weight is 101 kD, on western blots Chromator is detected as a doublet of approximately 130 kD, which may reflect post-translational modification(s).^{39,44} RNAi depletion of Chromator in S2 cells leads to microtubule spindle abnormalities and chromosome segregation defects,³⁹ consistent with a role for Chromator in spindle assembly and/

or function. Spindle phenotypes in a *Chromator* null mutant animal could not be easily analyzed due to maternal Chromator product obscuring embryonic analysis and early lethality preventing analysis of third instar larval neuroblasts.³⁹ However, recently an EMS mutagenesis screen for new Chromator mutants identified new hypomorphic *Chromator* alleles that survive as transheterozygotes to the third instar larval stage.⁴⁵ Analysis of larval neuroblast spindles in these animals revealed similar severe spindle and chromosome segregation defects⁴⁶ confirming that Chromator is required for proper spindle function in the animal. However, Chromator also lacks any features characteristic of a structural or scaffolding protein.

Megator can be co-purified in a complex with Skeletor and Chromator.⁴⁰ In contrast to both Skeletor and Chromator, Megator with its long coiled-coil domain⁴⁷ does exhibit features consistent with a scaffolding function. Indeed, expression of the coiled coil domain alone, which lacks a nuclear localization signal, results in formation of self-assembling spherical structures in the cytoplasm.⁴⁰ Megator is a large 2,346 amino acid protein that migrates at approximately 260 kD and shows overall structural similarity to the mammalian nuclear pore complex protein Tpr⁴⁸⁻⁵⁰ and to the yeast nuclear pore complex-associated proteins

Mlp1p and Mlp2p.^{51,52} The N-terminal 70% of Megator is comprised of an extended coiled-coil region while the C-terminal 30% of the protein is unstructured and acidic.⁴⁷ Although this organization is conserved between Tpr and Megator, the sequence identity between the two proteins is only 28%. *Megator* maps to 48C5 on the second chromosome and a P-element insertion (*l(2)k03905*) generated from a large P-lacW insertional mutagenesis project⁵³ is present at the +1 position of the published Megator cDNA.⁴⁰ Western blot results suggest that this insertion is likely to be a null allele. Although early embryonic divisions appear normal due to maternal stores of wild-type *Megator* mRNA deposited by the heterozygous *l(2)k03905/+* mother, the vast majority of homozygous *l(2)k03905/l(2)k03905* offspring die during embryogenesis and the few that survive to first instar larvae die shortly after molting, confirming that *Megator* is an essential gene. Consistent with a role for Megator in mitosis, RNAi depletion of Megator in S2 cells leads to a lower mitotic index.^{40,54}

east (enhanced adult sensory threshold) maps to 2C2-4 on the X chromosome and was originally identified as a viable, semidominant P-element insertion that exhibited chemosensory defects.^{55,56} This P element was used to generate imprecise excision events to obtain additional hypomorphic and lethal *east* alleles.⁵⁷ Consistent with a role in spindle formation or function, loss-of-function *east* mutations lead to an increased frequency of mitotic errors as well as abnormal chromosome congression.⁵⁷ EAST is found in a complex with Megator⁴¹ and yeast two-hybrid interaction assays revealed a direct interaction with Chromator as well.⁴³ EAST is also a large protein of 2,362 amino acids that migrates as a 265 kD protein on SDS-PAGE. Apart from seven potential nuclear localization sequences, twelve potential PEST sites, and a signature zinc-binding region typical of zinc carboxypeptidases, EAST does not have any obvious domains or structural motifs.⁵⁷

Dynamic Redistribution of Spindle Matrix Proteins during Mitosis

The founding member of the spindle matrix complex, Skeletor, was identified by its striking dynamic redistribution pattern observed throughout the cell cycle (Fig. 2). The anti-Skeletor antibody mAb1A1 stains a fibrous meshwork that extends throughout the nucleus at interphase but as the nucleus enters prophase the staining begins to realign into a spindle-like structure as well as co-align with the nuclear envelope. By metaphase and anaphase Skeletor antibody labels a complete spindle-like

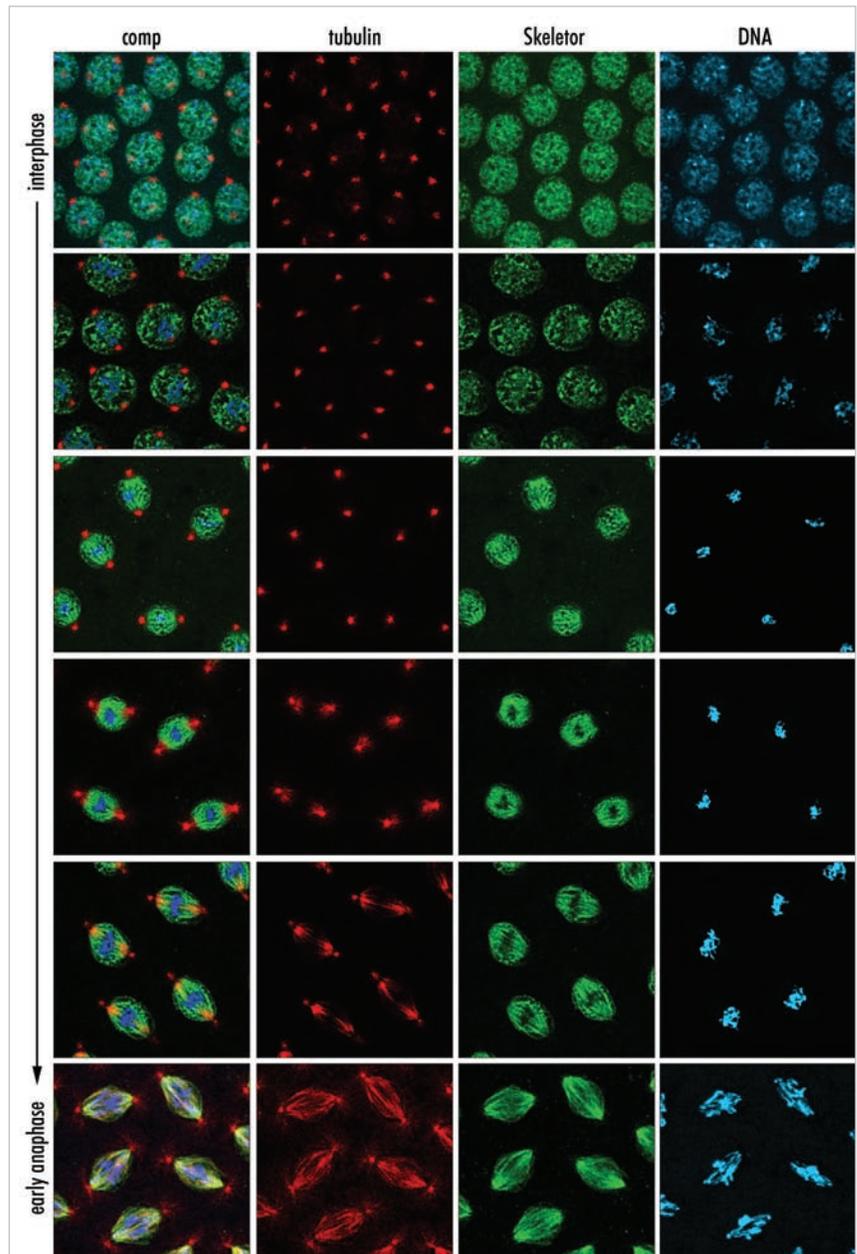


Figure 2. Dynamic distribution of the spindle matrix protein Skeletor relative to microtubules during the cell cycle. Confocal images of triple labelings of syncytial blastoderm nuclei using anti-tubulin (red) and anti-Skeletor (green) antibodies and Hoechst to visualize the DNA (blue). Top: during interphase Skeletor is found in the nucleus localized to the chromosomes while microtubules are excluded from the nucleus. (Panels 2–5) progression of the reorganization of Skeletor into a spindle matrix structure and microtubules into a microtubule spindle. During early stages of its reorganization Skeletor associates with the nuclear rim and shows less overlap with the DNA (Panel 2), subsequently forming a spindle-like structure before microtubules have invaded the nuclear space (Panel 3). As the microtubule spindle is established (Panels 4 and 5) it coaligns with the mid-region of the spindle matrix. During early anaphase Skeletor shows extensive overlap in the composite (comp) image with the microtubule spindle but is absent at centrosomes and on astral fibers.

structure throughout its entire length. This spindle-like structure persists in the mid-region during early telophase when the chromosomes begin to decondense but resolves back into a fibrous meshwork structure in each of the two daughter nuclei during late telophase. Closer analysis of the meshwork staining pattern

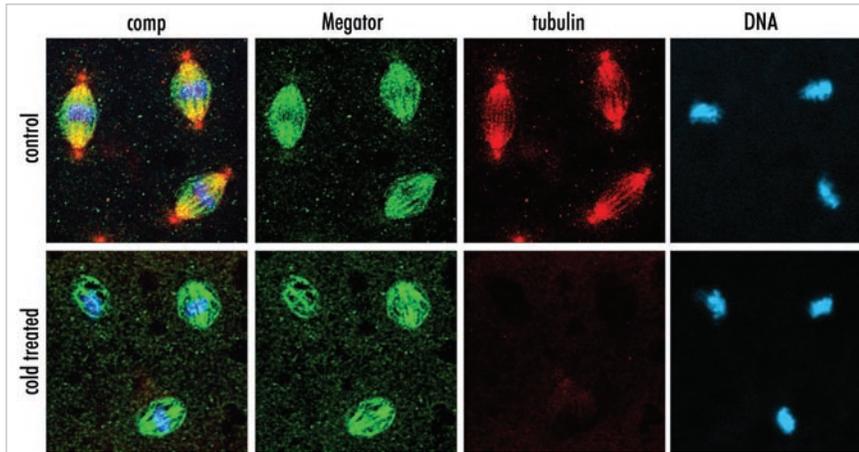


Figure 3. The spindle matrix remains intact after microtubule disassembly. Control (top) and cold-treated (bottom) embryos triple labeled with anti-Megator antibody (green), anti- α -tubulin antibody (red) and Hoechst (DNA in blue). In the cold-treated embryos the microtubule spindles have depolymerized, as indicated by the absence of microtubule labeling, whereas the Megator-stained spindle matrix remains intact, demonstrating that this structure persists independently of the microtubule spindle.

in embryonic nuclei revealed significant overlap with Hoechst-stained DNA³⁸ and Skeletor's localization to DNA during interphase was indicated by antibody labeling of interbands on polytene chromosomes (**Fig. 1B**).³⁹ Chromator is coincident with Skeletor staining at interphase and, like Skeletor, reorganizes into a spindle-like structure from prophase to metaphase (**Fig. 1B**). However, in addition to its colocalization with Skeletor, Chromator is also found on centrosomes and is prominent at the midbody during telophase.³⁹

Although both Megator and EAST colocalize with the Skeletor-defined spindle at metaphase,^{39,40} they are both part of an interchromosomal domain during interphase.^{39,40,47,57} Staining for both of these proteins is complementary to Hoechst staining of the DNA, easily visualized in salivary gland nuclei whole mount preparations where they fill the intranuclear space between chromosomes or in light squash preparations where they appear as “sausage casing” enwrapping the chromosomes (**Fig. 1B**).^{39,57} In addition, Megator also localizes to the nuclear rim, presumably as part of the nuclear pore complex.⁴⁷ The dynamics of how proteins from two different nuclear compartments (i.e., Skeletor and Chromator in a chromosomal compartment and Megator and EAST in the interchromosomal domain) reorganize during mitosis to come together into a spindle-shaped structure will require future live imaging studies. However, the association of a subset of Skeletor and Chromator with the nuclear envelope (**Fig. 2**) where Megator is localized during the early prophase could suggest that these two proteins disassociate from the chromosomes to align with the reorganizing Megator/EAST nuclear structure.

The microtubule spindle coaligns with the spindle-like structure comprised of Skeletor/Chromator/Megator/EAST during metaphase. However, several lines of evidence indicate that the Skeletor/Chromator/Megator/EAST structure is a separate entity from the microtubule spindle: (1) the formation of this structure precedes the formation of the microtubule spindle (**Fig. 2**); (2) this structure tends to extend more broadly than

the microtubule spindle (**Fig. 2**); and (3) this structure persists even after microtubules are disassembled with nocodazole-, colchicine- or cold-treatment (**Fig. 3**). Thus, different lines of evidence support that Skeletor, Chromator, Megator and EAST comprise part of a bona fide spindle matrix structure. Importantly, however, there is a physical relationship between the microtubule spindle and the spindle matrix since if microtubules are disassembled, the spindle matrix structure, though remaining intact, becomes more compressed.^{38,39,54} Likewise, if spindle matrix components are perturbed (e.g., in mutant animals or by RNAi) the microtubule spindle can become malformed and chromosome segregation can be impaired.^{39,46,59}

Spindle Matrix Function

The critical role of microtubules and motor proteins in spindle assembly and function is undisputed yet the complexities underlying spindle dynamics as well as the mechanics of force production suggest other players are likely to be involved. Thus the concept of a “spindle matrix” was initially proposed as a stationary or elastic structure or scaffold that interacts with and stabilizes the microtubule-based spindle during force production.¹⁷⁻¹⁹ In some formulations of the model the spindle matrix is also proposed to provide a motor system working in concert with microtubule motors.^{18,35,60} Indeed, the spindle matrix could be envisioned to perform multiple functions and some of the data obtained in flies support multiple roles for the spindle matrix, as reviewed below.

Microtubule assembly and function. The reorganization of spindle matrix components into a separate spindle-like structure that precedes, yet at metaphase coaligns with, the microtubule spindle infers a potential role for the spindle matrix to serve as a guide or stabilizing structure for the microtubule spindle.³⁸⁻⁴⁰ Fluorescence recovery after photobleaching (FRAP) experiments in S2 cells indicate that the spindle matrix is less dynamic than the microtubule spindle as measured by bleaching fluorescently-tagged Megator,⁵⁴ a result consistent with a scaffold function for the spindle matrix. In that same study, however, RNAi knock-down of Megator was correlated with defects in spindle maturation such as impairment of recruitment of Mad2 and Mps1 to unattached kinetochores affecting mitotic duration and spindle assembly checkpoint (SAC) response. However no obvious defects were observed in microtubule spindle assembly or shape. This could indicate that undetectable levels of Megator remaining after the RNAi treatment are nevertheless sufficient for function or that Megator is functionally redundant with other matrix component(s). When the spindle matrix is instead perturbed by expression of mutant spindle matrix components consisting of early truncation or a missense substitution in Chromator and Megator, respectively, the microtubule spindle is observed to show a range of abnormalities including mis-aligned and/or multipolar spindles.^{46,59}

Force production. Even after a UV microbeam was used to sever k-fibers during anaphase in crane fly spermatocytes, chromosomes continued to be pulled to the spindle poles.^{19,34,35} These results suggested that poleward forces are acting *on* the k-fibers that themselves act to resist these forces, and the rate of chromosome movement to the poles might, in fact, be regulated by the rate of k-fiber disassembly.^{36,61} It has been proposed that a separate system, the spindle matrix, contributes to these pulling forces.⁶⁰ Although similar experiments at anaphase have not yet been performed in *Drosophila*, the spindle matrix does appear compressed after nocodazole- or cold-treatment.^{38,40} This compaction is best observed in timelapse imaging experiments of mCherry-tagged Megator upon colchicine-induced depolymerization of the microtubules.⁵⁴ Such results would be consistent with the presence of compressive forces within the matrix.

Chromosome dynamics. Irrespective of whether the spindle matrix is directly responsible for force generation, it appears to play a role in proper chromosome congression and/or segregation. Loss or mutation of *east* hinders chromosome congression to the metaphase plate, delays the onset of anaphase, and leads to nondisjunction of achiasmatic chromosomes in female meiosis.⁵⁸ Conversely, although depletion of Megator by RNAi in S2 cells also interferes with chromosome congression leading to a poorly defined metaphase plate, anaphase onset occurs prematurely and cells progress through mitosis ~15% more rapidly than normal.⁵⁴ Interestingly, Megator RNAi-induced errors in chromosome congression were ameliorated when anaphase onset was delayed by treating the cells with the proteasome inhibitor MG132.⁵⁴ RNAi depletion of Chromator in S2 cells or a hypomorphic *Chromator* mutant allele also perturbs chromosome segregation as indicated by a high incidence of misaligned and/or lagging chromosomes on the spindle.^{39,46}

Regulation of the spindle assembly checkpoint. The spindle assembly checkpoint ensures proper chromosome segregation by inhibiting the anaphase-promoting complex (APC) to delay anaphase until all of the kinetochores (KTs) are properly attached to spindle microtubules.^{62,63} The presence of activated Mad2 in a checkpoint complex at unattached KTs serves as the primary checkpoint sensor. In addition, spindle defects activate the kinase Mps1 which hyperphosphorylates Mad2 to trigger metaphase arrest.^{64,65} The lower mitotic index and weakened checkpoint response to MT depolymerization found in Mtor-depleted cells^{40,54} is consistent with a requirement for Mtor for proper SAC response. Furthermore, Lince-Faria et al.⁵⁴ also showed that the premature anaphase onset observed in Mtor-depleted S2 cells was very similar to that observed after depletion of the spindle assembly checkpoint protein Mad2. Consistent with this, a subset of Mad2 accumulates at the nuclear-derived spindle matrix during mitosis when microtubules just begin to invade the nuclear space and is retained in this structure even after microtubule depolymerization. Confirmation of a physical interaction was shown by immunoprecipitation experiments that revealed Mtor interacts in a complex with both Mad2 and Mps1. Depletion of Mtor results in loss of Mad2 from unattached KTs.⁵⁴ Taken together, these results are consistent with a role for the spindle matrix as a spatial regulator of SAC signaling, facilitating the activation and recruitment of Mad2 and Mps1 to unattached KTs as cells enter mitosis.

This role appears to be evolutionarily conserved, as the mammalian Mtor ortholog, Tpr, also interacts with Mad2 and is required for checkpoint signaling.^{54,66} Interestingly, the spindle matrix may sequester other signaling molecules to the spindle region as well during mitosis. Recently Mtor was shown to physically interact with Asator, the *Drosophila* tau-tubulin kinase homolog that is cytoplasmic during interphase but localizes to the spindle during mitosis.⁶⁷

Other possible functions. Early studies in *Drosophila* noted a mitotic membranous “spindle envelope” that included lamin B that was proposed to act to confine proteins to the spindle region to promote spatio-temporal regulation of spindle assembly factors before anaphase.^{3,68-71} However, MT disassembly experiments reveal exchange of a microtubule-associated protein, Jupiter, occurs with the cytoplasm despite the presence of this spindle envelope, arguing that it does not act as a diffusion barrier⁵⁴ which is consistent with EM imaging studies showing that this membrane is fenestrated.⁶⁸ Experiments in other systems suggest lamin B and a membranous spindle matrix may tether spindle assembly factors⁷² as well as serve to partition membrane systems during cell division.⁷³ Though these functions have not yet been explored in *Drosophila*, the rapid mitotic cycling in embryonic nuclei that would presumably be greatly facilitated by spindle assembly factor sequestering suggest *Drosophila* should be a good model system to elucidate such potential roles for the spindle matrix. In addition, it is possible that much like chromosomal passenger proteins that “piggyback” on chromosomes during mitosis,^{74,75} there may be cellular components that for structural reasons are difficult to degrade and reassemble on a rapid time scale that therefore associate with the spindle matrix during division to ensure equal partitioning to daughter cells during division.

Evolutionary conservation. The apparent lack of evolutionary conservation of Skeletor, Chromator and EAST with spindle proteins in other eukaryotes has raised the issue of the general significance of these proteins.⁷⁶ However, multiple studies spanning the evolutionary spectrum from lower eukaryotes to vertebrates have provided intriguing evidence that a spindle matrix may be a general feature of mitosis.⁸ Nonetheless, considering the diversity of potential and unrelated spindle matrix molecules so far implicated the possibility that different molecules may have attained the same function in different organisms. Alternatively, this could reflect the presence of multiple redundant systems or that different spindle matrices are operational at different stages of the cell cycle. Therefore, if the principles for how a stationary or elastic molecular matrix independent of microtubule polymerization can contribute to microtubule spindle function and/or assembly could be established in a model system such as *Drosophila*, it would be likely to inform the function of other spindle matrices regardless of their specific molecular composition. And for at least one of the *Drosophila* spindle matrix proteins, Megator, functional orthologs have been identified in organisms from yeast to humans.⁸

Spindle Matrix Through the Cell Cycle: Double Duty at Interphase?

The spindle matrix components Skeletor, Chromator, Megator and EAST reside in two different interphase nuclear compartments,

the chromosomal and interchromosomal domains, before coming together at mitosis to form the spindle matrix. Several lines of evidence suggest they play important roles in their interphase compartments as well. Skeletor and Chromator localize specifically to regions within the interbands in a pattern coincident with the histone H3S10 kinase JIL-1,^{39,77,78} which has been shown to play a role in establishing euchromatin by preventing its packaging as heterochromatin.^{79,80} Chromator directly interacts with the C-terminus of JIL-1 although this interaction is not required for the chromosomal targeting of either protein.⁴⁵ However, loss of either protein severely perturbs polytene chromosome structure, resulting in not only the loss of interbands but also in disorganization of the banded regions.^{45,81,82} These results raise the prospect that one or more spindle matrix components (the effect of loss of Skeletor is not yet known) may play an important interphase role regulating genomic organization. Importantly, the observed disruption of chromosome structure is correlated with decreased gene expression levels in mutants of both *JIL-1* and *Chromator*.^{46,83} Consistent with a requirement for Chromator for proper regulation of gene expression, novel pupal-lethal *Chromator* mutants have been identified that cause developmental defects in muscle remodeling during metamorphosis and from these studies it was concluded that Chromator activates processes and genes involved in tissue destruction and remodeling.⁴³ These alleles do not show any changes in the Chromator coding sequence but consist of internal deletions of the P-element insertion that is present in the lethal loss-of-function *Chro*^{KG3258} allele³⁹ and thus are likely to be mutations that affect either the level and/or tissue-specific expression of the Chromator protein.

Although spindle matrix components sort into two different compartments (chromosomal and interchromosomal domain) at interphase, they still functionally interact. In their study of muscle remodeling during metamorphosis, Wasser et al.⁴³ found that EAST is also involved in tissue destruction and remodeling events during development and, intriguingly, acts to counteract the activity of Chromator. So whereas *Chromator* mutants showed incomplete muscle histolysis, the muscles of *east* mutants degenerated prematurely. Additional support for an antagonistic role for these two proteins includes that eclosion defects of pupal-lethal *Chro* alleles are rescued in an *east* heterozygous background.⁴³ Thus, EAST appears to inhibit the same processes and genes involved in tissue destruction and remodeling that are activated by Chromator although the precise mechanism for this regulation is not yet well understood. Despite that the majority of interphase EAST resides in the interchromosomal domain, it is possible that a subset of EAST not easily detected in whole mount stainings does interact with chromosomes to regulate gene expression. For one, FRAP experiments indicate that whereas in non-permeabilized nuclei the interchromosomal EAST-GFP is highly mobile, when nuclei are permeabilized under low salt conditions, EAST-GFP rapidly associates with chromosomes and mobility significantly decreases. However, if EAST's C-terminus, which includes the Chromator interaction domain, is truncated, EAST- Δ CTD-GFP's affinity for chromosomes is severely reduced and mobility recovers to normal levels even after exposure to permeabilized, low salt conditions.⁴³ Notably, when EAST-GFP was observed to associate with chromosomes, it localized to transcriptionally silent heterochromatic regions, which would further support a role in gene repression.⁴³

Other studies also have supported a role for EAST in regulating gene expression.^{84,85} Increased levels of EAST protein were found to nearly completely repress *yellow* expression at the *y*² allele in a manner dependent on the *MDG4* retrotransposon sequences present in this allele. It was suggested that this effect may be due to a role for EAST in regulating insulator activity and/or gene positioning in the nucleus.⁸⁴ A subsequent study, however, showed further examples of the effect of manipulating EAST levels on regulation of additional candidate genes. In this case, some genes that contained *MDG4* sequences showed no effect, while other genes without *MDG4* sequences were repressed.⁸⁵ Thus the authors speculate that EAST is part of a global protein system of the nuclear matrix that may function to interact with chromosomal factors that regulate proper temporal and tissue-specific expression of all genes.⁸⁵ An alternative model proposes that EAST may act as a salt sensor that modulates gene expression in response to changing ion (Na^+/K^+) concentrations with lower salt conditions mediating binding to chromosomes and repression while higher salt conditions trigger dissociation from chromosomes and activation of target genes.⁴³ Since the cast of chromatin characters present influences ionic effects felt at different genes, this could explain the inability to correlate EAST regulation of gene expression with a specific genomic sequence or insulator protein.⁸⁵

Megator, which colocalizes in the interchromosomal domain at interphase with EAST, has also been associated with regulation of gene expression at interphase. The MSL dosage compensation complex copurifies with Megator and depletion of Mtor was reported to eliminate targeting of the MSL complex to the male X.⁸⁶ Proper localization of the MSL complex is a required step to achieve the dosage compensation that equalizes the level of X-linked gene expression in males and females (reviewed in ref. 87). Megator's role in this process is not clear, but several models have been proposed including that Megator may influence dosage compensation through chromatin loop organization and spatial positioning at the nuclear pore complex. Alternatively, Megator might link transcription to the regulation of nuclear export of mRNAs through the nuclear pore complex by the exosome. In the third model Megator might function away from the nuclear pore complex in the interchromosomal domain to bind chromosomal regions to activate or repress gene expression.⁸⁸ It has also been suggested that Megator might mediate compartmentalization of the X chromosome that facilitates the targeting and/or spreading of the MSL complex.⁸⁹

In summary, the interphase functions of spindle matrix components promise to be as fascinating and significant as their mitotic functions. Although nuclear architecture has traditionally been considered to "disassemble" during mitosis, it may be more accurate to characterize it as being "remodeled" throughout the cell cycle. This provides the cell with a malleable structure that may facilitate nuclear or spindle architectural organization as well as provide a binding platform for regulatory and other factors that contribute to cell cycle specific functions such as gene regulation or chromosome segregation.

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