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Mini-Review

Kinesin-14 leaps to pole position in bipolar spindle assembly

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Formation of a mitotic spindle occurs almost effortlessly as cells cycle and is essential for chromosome segregation. Early in a cell cycle, the replication of centrosomes or spindle pole bodies at G_1/S leaves mother and daughter poles in close association until forced apart into a bipolar arrangement at mitotic onset. Kinesin-like proteins (Klps) generate a variety of forces that contribute to the timing, formation and maintenance of spindle bipolarity. The ability of two key players, Kinesin-5 and Kinesin-14, to cross-link both parallel and anti-parallel microtubules has led to emphasis on spindle microtubule interactions in the spindle assembly mechanism. Recent identification of a Kinesin-14 binding site on γ-tubulin, a ubiquitous component of microtubule organizing centers (MTOCs) at poles combined with the ability of changes to MTOC complexes to regulate bipolarity, now shifts an inquisitive eye to pole mechanisms and to the complexities evolving around MTOCs.

The ability of cells to dynamically transform the microtubule cytoskeleton creates powerful flexibility. Chromosome segregation makes use of such a temporally fleeting structure, the mitotic spindle. Walter Flemming defined mitosis in 1882 as the formation of paired threads of chromatin. Mitosis has been sketched, imaged, filmed, modeled and prodded now for more than 126 years, yet it is only over the past two decades that we are beginning to understand one of its most fundamental aspects-spindle assembly. Perhaps most surprising is that despite the essential need to assemble this structure preceding cell division, no one overriding mechanism exists. Nor have all the rules been defined. Key components include centrosomes, or spindle pole bodies, and their microtubule organizing center (MTOC) complexes, microtubule nucleation, dynamics, organization and attachment, microtubule motor and bundling proteins, and chromatin. When centrosomes are present, they dominate spindle formation, however microtubules can be nucleated in the absence of centrosomes around chromatin and focused at minus-ends by the cross-linking and sliding activity of microtubule motor proteins. In the latter case, mathematical modeling of limited parameters can provide experimentally testable insights,[1,2] however much is unknown to effectively model all aspects of spindle assembly.[3] As well exciting new findings and concepts continue to modify existing themes.[4-7]

The dynamics of spindle formation and chromosome segregation suggested roles for microtubule motor proteins even before the discovery of conventional kinesin in 1985. This was followed by an explosion of kinesin-like proteins (Klps), also referred to as kinesin-related proteins (Krps) or kinesin superfamily proteins (KIFs), beginning in 1990.[8] Greater than 300 Klps are now known, from a variety of eukaryotes. Those best characterized are classified into fourteen families, plus an orphan group, with universal names Kinesin-1 through Kinesin-14.[9] Conservation of mechanisms had been observed and kinesins classified previously,[10,11] but the use of uniform nomenclature in 2004 even further emphasized cross-species similarities in function and localization. Not all fourteen Klp families are represented in each eukaryote, nor are all required for spindle function. Two Klp families among the first identified, are consistently present, Kinesin-5 and Kinesin-14, and known to regulate bipolar spindle assembly. In many organisms Kinesin-5 is essential for establishing bipolarity, generating monopolar spindles with side-by-side poles when it fails. Kinesin-14s oppose spindle bipolarity, antagonizing the action of Kinesin-5. The ability of both of these kinesin families to cross-link microtubules, as well as opposite directionality of movement on microtubules, has led to emphasis on these aspects in models not only for maintenance but for the establishment of spindle bipolarity. For Kinesin-14s microtubule cross-linking, sliding and focusing need not be its sole mechanism for regulating spindle assembly[12-14] and has not been shown to be the primary mechanism in the presence of centrosomes and spindle pole bodies in eukaryotes. In multi-cellular eukaryotes a distinct minus-end directed microtubule motor protein, dynein, also has visible roles in pole focusing.[15] In contrast to Kinesin-5 or dynein, extensive genetic evidence across multiple species indicates overlap of Kinesin-14 function with pole proteins, either in centrosome stability[16,17] or interactions with MTOC proteins that form the site of nucleation and attachment of microtubule minus ends,[18-21] or in regulation of microtubule number and length from poles.[22]

The difficulty in deciphering precise spindle assembly mechanisms lies in part with fundamental gaps that remain in our knowledge of mitotic components, including motor proteins and spindle pole

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macromolecular assemblies. Centrosome structure and organization is more complex than that of yeast spindle pole bodies, but both use MTOCs as sites of microtubule nucleation and attachment.[6,23] Still MTOC proteins have not yet been extensively characterized in any species. In regard to Klps, the motor domains of several Klps have been crystallized,[24,25] however the nonmotor stalk domains in Klps remain poorly characterized. Limited sequence similarities are present in stalk domains of Klps, even within families, to assist identification of regions important in protein-protein interactions or cellular regulation. Klp binding to tubulin, both the tubulin binding site and the interacting surface domain of the motor are just beginning to be understood.[4,26] Continuous advances in technology have led to tremendous information available through structural analysis of Klp/tubulin interactions, high resolution imaging by electron microscopy and tomography, in vivo dynamic time lapse imaging and single molecule studies and are complemented by the often unpredictable surprises that genetic analysis can reveal.

In Rodriguez et al.,[4] exciting discoveries are made that challenge some basically held concepts in current models of the role of Kinesin-14 Klps and their regulation of bipolar spindle assembly. Genetic analysis in several eukaryotes has continued to point to overlapping roles for Kinesin-14 Klps and γ-tubulin. Discovered in 1989, not long after Kinesin, this specialized tubulin localizes to the minus ends of microtubules at spindle poles.[27] As part of MTOCs, γ-tubulin functions as an adaptor protein for the α/β-tubulin heterodimers that assemble into microtubules of the spindle. Gamma-tubulin binding proteins contribute to form the core of the MTOC and were identified first in budding yeast, then found to be components of eukaryotic MTOCs in human, Drosophila and in fission yeast.[27,28] Perhaps in no other eukaryote is the MTOC so widely utilized as in fission yeast, with interphase (iMTOC) and equatorial (eMTOC) complexes present in addition to the spindle pole MTOCs. Each must be temporally activated around the mitotic MTOC. Several conditional alleles exist for MTOC components in fission yeast and can be subclassified into a group that exhibits strikingly similar effects on microtubule dynamics and organization.[19,21,29] It is this group that appears to have prominent affects on bipolar spindle assembly in fission yeast, as well as affects on non-mitotic MTOCs that may be informative. Rodriguez et al. found that these selected alleles of all core MTOC proteins, Alp4, Alp6 and γ-tubulin when individually combined with a compromised allele of Kinesin-5 Cut7, *cut7-22^{ts}*, rescued spindle bipolarity. Two of these alleles when combined, *gtb1-PL302* and *alp4-1891*, co-suppress their single allele microtubule phenotypes.[29] When this occurs, the combined effectiveness to rescue bipolarity is also reduced. Thus the interaction between Alp4 and γ-tubulin changes aspects of microtubule stability and organization that impacts spindle bipolarity. These pole-initiated effects can override the antagonistic mechanism of Kinesin-14 Pkl1 in opposing bipolarity in the *cut7-22^{ts}* strain.

Kinesin-14 Klps in both budding yeast and fission yeast have been shown to regulate microtubule dynamics and organization, but not aspects of microtubule nucleation. In fission yeast genetic links of Kinesin-14 Pkl1 with all MTOC proteins, suggests an intimate relationship with the complex. The γ-tubulin allele *gtb1-PL302* was initially identified in a synthetic lethal screen for mutations that confer dependence on the normally nonessential Kinesin-14 Pkl1 for viability.[19] Exciting work by Niwa and Toda revealed additional links between MTOC proteins Alp4 and Alp6 and Kinesin-14 Pkl1.[29] Such compelling genetic associations begged further investigation. Can Kinesin-14s bind directly to γ-tubulin and if so with what impact? Can such an interaction generate changes to microtubule organization or dynamics that regulate bipolar spindle assembly? In fission yeast Kinesin-14 Pkl1 was found to associate with γ-tubulin and MTOC proteins in vivo and Pkl1p binds directly to γ-tubulin through its motor domain by yeast two-hybrid. To determine how this interaction contributes to the regulation of bipolar spindle assembly in fission yeast, perhaps in addition to microtubule interactions, Rodriguez et al. needed to selectively disrupt Kinesin-14 Pkl1 binding to γ-tubulin while leaving the Kinesin-14/microtubule association intact. Since no Klp binding site on any tubulin had previously been defined this required first defining such a domain on tubulin. To do this for γ-tubulin, conservation of mechanism with Klp binding to β-tubulin of microtubules was assumed.

Structural characterization of the docking of Klps to microtubules, based on higher resolution structures of the α/β-tubulin heterodimer, conventional kinesin and Klps, currently provides some of the best available experimental data for motor protein interactions on tubulins.[24,25] Crystallographic images from docking different eukaryotic dimeric Klps to microtubules indicate similar interactions on mammalian tubulin. Conserved and recognizable features of the tubulin surface must therefore be present across eukaryotes. Dimeric Klps are observed to dock along the protofilament near paired helices, numbered 11 and 12, on β-tubulin, but not with similarly positioned helices on adjacent α-tubulin. Analysis by Rodriquez et al. revealed little sequence variation in helix 11 of β-tubulins across species and requisite limited similarities when compared with α-tubulin consistent with crystallographic images of dimeric Klps bound to microtubules. Within γ-tubulins, a subset of β-tubulin helix 11 residues are conserved and create a consensus, LFK/Er…Q. Structural modeling indicates that lysine (K) and glutamine (Q) side chains are most accessible to protein-protein interactions, and indeed were found to be components of a Kinesin-14 Pkl1 binding site on γ-tubulin. Altering either K or Q positions to alanine impaired Kinesin-14 Pkl1 binding to γ-tubulin and both mutations alone were sufficient to relieve Kinesin-14 Pkl1 opposition to spindle bipolarity. Thus changes to the MTOC complex, either by altering the Alp4/γ-tubulin interaction or the Kinesin-14 Pkl1/γ-tubulin interaction, can override opposition to spindle bipolarity, each despite the continued presence of Kinesin-14 Pkl1 on β-tubulin of microtubules.

The work by Rodriguez et al. raises exciting questions for the role of Kinesin-14s in regulating the mechanism of spindle bipolarity. In addition to γ-tubulin, Kinesin-14 Pkl1 also interacts directly with MTOC proteins Alp4 and Alp6 by yeast two-hybrid analysis (unpublished findings). How such interactions affect all or a subset of MTOC functions is unknown. Subtle alterations in binding of Alp4 to γ-tubulin in the MTOC can lead to dramatic consequences as revealed by the *gtb1-PL302* allele that strengthens this interaction. Structural analysis will continue to play a vital role in understanding protein interactions and the crystallization of single or multiple MTOC proteins to help visualize the multi-protein complex is certain to be a milestone to our understanding of its mechanisms as it has been for others.[30] In regard to Klp/tubulin binding, the conservation of only a subset of helix 11 residues shared between β- and γ-tubulin raises the intriguing possibility that selectivity for

a subset of Klp interactions exists at poles. We are investigating the Klp/microtubule binding site by generating comparative changes to β-tubulin helix 11 in microtubules and examining the functional compatibility of this domain by switching this region between β-tubulin and γ-tubulin. As well we are exploring compatibility of Kinesin-14 function across species using the well characterized Drosophila and HSET Kinesin-14 Klps in fission yeast to determine if they can replace Kinesin-14 Pkl1 functions in part or entirety, particularly in an MTOC mechanism. A complete view of spindle assembly must begin to incorporate knowledge of MTOC mechanisms.[6,23,28] In fission yeast, work on cellular MTOCs is poised to undertake the challenge.

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