humans heterozygous for Rb chromosomes can incur fitness costs in the form of reduced fertility [17,18]. This sets up an intriguing tension in populations, in which Rb chromosomes are propagated because of their female meiotic advantage but are impeded because of the associated fitness disadvantages. Such fitness tradeoffs are likely to rapidly select for modifiers that act in centromere specification or meiosis to alleviate the fitness disadvantage. Indeed, many essential proteins involved in both these processes evolve rapidly between species, hypothesized to be a result of such recurrent cycles of female meiotic drive and suppression [14,16]. Thus, lowered fitness costs due to selection of modifiers could allow fixation of Rb chromosomes in certain populations. In these situations, hybrids between populations fixed for different Rb chromosomes could unleash deleterious effects in meiosis, resulting in chromosomal speciation [19].

The results of Chmatal *et al.* highlight the insight that can be revealed by cell biological approaches to old evolutionary questions. The establishment of Rb chromosomes as a cell biological model opens up the possibility of further insight into another poorly studied but necessary determinant for female meiotic drive — asymmetry of the first meiotic spindle in oocytes. It is this asymmetry that must be exploited by ‘cheating’ meiotic drivers. How this asymmetry is established, and how it can be exploited is practically unknown. Early studies in the grasshopper *Myrmeleotettix maculatus* found that the meiotic spindle was asymmetric — fibers from the egg pole to the equator were measured to be approximately three times as long as those from the polar body pole [20]. Taking advantage of this asymmetry, B chromosomes in this species drive by positioning themselves on the eggward side of the spindle. Similar to their insights into centromere strength, driving Rb chromosomes may help further dissect the mysterious cell biology of female meiosis in animal oocytes.

Even Mendel might have approved.

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http://dx.doi.org/10.1016/j.cub.2014.08.053

Mitotic Kinesins: A Reason to Delve into Kinesin-12

The failure of kinesin-targeting cancer drugs is thought to result from functional redundancy of mitotic kinesins. A new study provides mechanistic insights into kinesin-12 that help to explain its targeting to kinetochore fibers and its ability to compensate for inhibition of kinesin-5.

William O. Hancock

The intricate dynamics of mitotic spindle morphogenesis involves many proteins that possess overlapping functions. While this redundancy is natural, given the vital importance of faithfully separating duplicated chromosomes, it hampers efforts toward a detailed understanding of spindle dynamics. Further, because targeting the mitotic spindle is an attractive approach for anti-tumor therapeutics, this functional redundancy reduces the probability of finding effective single-target drugs. One promising target is kinesin-5 (KIF11 or Eg5), a tetrameric kinesin that plays a key role in spindle formation by generating forces that separate the two poles. In cell culture, inhibition of Eg5 results in monopolar spindles and mitotic arrest [1]. The trouble is that in clinical trials, Eg5 inhibitors are less effective than hoped, and a principal reason is thought to be this problem of redundancy — other

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motors taking over the function of Eg5, allowing cells to escape mitotic block [2].

The kinesin-12 motor KIF15 is thought to share some properties of Eg5, making it a potential target for a combination therapy with Eg5. Cells are able to complete mitosis if Eg5 is inhibited after spindle formation, but inhibiting both Eg5 and KIF15 results in spindle collapse and mitotic arrest [3,4]. In human cells, KIF15 overexpression confers the ability to undergo cell division following pharmacological disruption of Eg5, providing a clue as to how tumor cells may develop resistance to Eg5 inhibitors [5]. These studies have provided significant motivation to understand (*to delve into*) kinesin-12 (KIF15), and in this issue of *Current Biology*, Sturgill, Ohi and colleagues uncover important mechanistic details of KIF15 that help to understand its role in spindle formation and maintenance, as well as how it may functionally replace Eg5 under some conditions [6].

In mitotic cells, KIF15 localizes to K-fibers, bundles of microtubules that span between the kinetochore and the spindle poles [3,4]. Because these K-fibers are the mechanical elements through which poleward forces on duplicated chromosomes are exerted, they are crucial to the delicate force balance that exists in the mitotic spindle. KIF15 knockdown causes a decrease in spindle length, suggesting that KIF15 may elongate K-fibers by sliding parallel microtubules relative to one another [3,5].

The new study by Sturgill *et al.* requires a reevaluation of the mechanism by which KIF15 localizes to K-fibers. Until now, the consensus in the field has been that KIF15 localizes to K-fibers through its interactions with TPX2 (Targeting Protein for Xklp2, the *Xenopus* KIF15 ortholog) [3,5]. In support of this idea, TPX2 is in the nucleus in interphase when KIF15 does not localize to cytoplasmic microtubules, and following nuclear envelope breakdown, TPX2 localizes first to K-fibers, followed by KIF15 localization [3,5]. Furthermore, TPX2 knockdown eliminates KIF15 spindle localization [3]. However, evidence is lacking for a direct interaction between KIF15 and TPX2 in the absence of microtubules [3,6,7].

In their new work, Sturgill *et al.* propose an alternative explanation for the K-fiber localization of KIF15 [6]. Instead of localizing through interactions with TPX2, they conclude that KIF15 naturally localizes to microtubule bundles because it possesses both a secondary (non-motor) microtubule binding domain and an autoinhibitory domain. Structurally, KIF15 can be subdivided into three domains: the heads, the proximal coiled-coil (coil-1) and the distal coiled-coil (coil-2). Their model is that the motor is normally autoinhibited by coil-2, but in microtubule bundles the motor becomes activated such that the heads bind one microtubule and coil-1 binds a second microtubule, resulting in relative sliding of the microtubules in the bundle.

In support of this autoinhibition mechanism, they show in interphase cells that deleting coil-2 causes the motor to localize to interphase microtubules, and *in vitro* the addition of an antibody that binds to coil-2 results in robust processive motility. Finally, hydrodynamic analysis suggests the motor can transition between a compact and an extended form. These results all point to coil-2 acting as an autoinhibitory domain, akin to the tail domain of kinesin-1 [8].

Sturgill *et al.* also identify a non-motor microtubule-binding domain in KIF15, with the surprise being that instead of it being located at the carboxy-terminal tail of the motor like other kinesins [9,10], it is located in coil-1, between the motor domains and the autoinhibition domain. Supporting this contention, isolated coil-1 binds microtubules in pelleting assays, and a construct in which the autoinhibitory coil-2 domain is deleted bundles microtubules *in vitro* in the absence of ATP and slides microtubules relative to one another in the presence of ATP.

To address the question of why KIF15 localizes to K-fibers in cells, Sturgill *et al.* generated microtubule bundles *in vitro* using a completely different protein, PRC1, which crosslinks antiparallel microtubules into bundles [11]. Full-length KIF15 strongly labels PRC1-mediated microtubule bundles at moderate motor concentrations and walks processively along the bundles at single-molecule motor concentrations. Complementing this *in vitro* characterization, in mitotic cells lacking K-fibers, full-length KIF15 localized to pharmacologically-induced non-K-fiber microtubule bundles. Thus, their model is that KIF15 autonomously targets microtubule bundles and TPX2 is involved in forming or maintaining the bundles rather than direct recruitment of the motor.

While this work provides an important dissection of the different functional domains of KIF15, there are a number of remaining questions. The first question involves the mechanism of microtubule sliding. The best understood model is tetrameric Eg5, which slides antiparallel microtubules apart by virtue of its two pairs of motor domains located at each end of the molecule [12]. One might ask: why don't both pairs of heads just bind to the same microtubule? In other dimeric kinesins the tail and the heads do bind to the same microtubule, resulting in enhanced motor processivity [9,10]. In Eg5, the answer appears to be that the stiff tetrameric coiled-coil prevents the two pairs of heads from binding to the same microtubule [13]. But what about KIF15? Sequence analysis and electron microscopy suggest that KIF15 has a long discontinuous coiled-coil with a hinge between coil-1 and coil-2. Previous studies of PRC1-generated microtubule bundles found that inter-microtubule distances in them are roughly 35 nm [11]. By sequence analysis, this is well within the length of KIF15 between the heads and the end of coil-1. Perhaps the reason that the second microtubule binding site in KIF15 is located in coil-1 rather than at the end of coil-2 is that this central position provides steric constraints that prevent binding to the same microtubule to which the heads are bound, while a distal position would allow no such constraints.

The second question involves the mechanism of regulation. In kinesin-1, the motor is proposed to be activated by cargo binding to the tail domain, which relieves autoinhibition [8]. Sturgill and colleagues favor a mechanism by which there is an equilibrium between the open (activated) and closed (inhibited) conformations of the motor that under normal conditions is shifted strongly towards the inhibited state. At the high local microtubule concentrations found in microtubule bundles, microtubule binding (by the

heads or coil-1 or both) is proposed to lock the motor in an open (activated) conformation. Thus, this is a case of conformational selection rather than induced fit [14]. A more complete understanding of KIF15 regulation will require further tests of the precise inhibition mechanism as well as exploration of other modes of regulation such as phosphorylation or binding of coil-2 by activating proteins.

Before accepting all of the conclusions of Sturgill *et al.*, the experimental results must be reconciled with a recently published study of KIF15 from the McAinsh lab that used similar approaches but arrived at starkly different conclusions regarding the quaternary state of KIF15 [7]. Using native gels, gel filtration and photobleaching, Dreschler *et al.* found that KIF15 tetramerizes in an ionic strength and concentration-dependent manner, while Sturgill *et al.* used gel filtration, ultracentrifugation, electron microscopy and photobleaching to show that KIF15 exists as a dimer. While experimental conditions differed to some extent, there is no simple way to account for the dimer/tetramer discrepancy between the two studies. This is no small point because the different structures lead to contrasting interpretations of how KIF15 might generate inter-microtubule forces in cells. Another difference between the studies was the character of the motility. Sturgill *et al.* observed unidirectional single-molecule processivity and robust microtubule sliding when the motor was activated either by truncating coil-2 or by a coil-2 antibody. In contrast, in single-molecule assays Dreschler *et al.* observed runs in both directions as well as numerous pauses, and observed no clear sliding between pairs of microtubules. Based on the Sturgill work, this lack of microtubule sliding may result from coil-2 inhibiting motor activity. A conservative conclusion from theses studies is that KIF15 is able to tetramerize under some conditions, but a simple dimer is sufficient to generate microtubule sliding. Resolving these discrepancies should be at the top of the experimental to-do list.

Another intriguing question is what KIF15 is actually doing in K-fibers, or more specifically: can KIF15 slide apart both parallel and antiparallel microtubules? K-fibers are thought to be bundles of parallel microtubules with their minus-ends located at the poles and plus-ends abutting the kinetochore [15]. A simple explanation for why knockdown of KIF15 results in shorter spindles is that KIF15 elongates K-fibers [3,5]. However, this is a difficult geometrical problem. Antiparallel microtubule sliding makes geometric sense because forces generated by motors on opposite microtubules are additive. However, the mechanism by which a motor that spans two *parallel* microtubules could reliably slide one with respect to the other is more perplexing. If one microtubule has more motors bound to it through their tail domains, this would produce a net sliding force between the microtubules. But without this bias, motors on opposing microtubules should generate equal and opposing forces, resulting in zero net sliding force.

Relevant to this, Sturgill *et al.* observed sliding in microtubule pairs, but the relative microtubule orientations were not specified. Drechsler *et al.* claimed that in the rare events when unidirectional sliding was observed the activity represented parallel microtubule sliding, but because the microtubule orientations were not verified the result is not convincing. This question of parallel versus antiparallel microtubule sliding was addressed in detail by the Diez group for the kinesin-14 motor ncd, which also possesses a non-motor microtubule binding site in its tail [16]. By carefully identifying microtubule polarities, they clearly showed that ncd can robustly slide apart antiparallel microtubule pairs, but parallel microtubules only slide short distances before being locked together by motors bridging the two filaments. This type of investigation needs to be undertaken for KIF15.

What seems clear is that KIF15 possesses both a non-motor microtubule binding domain and an inhibitory domain, and due to this modularity it can autonomously target microtubule bundles such as K-fibers. While we await development of small molecule inhibitors of KIF15 that can be tested in combination with Eg5 inhibitors, these mechanistic insights into KIF15 will further our understanding of how motors, microtubules and regulatory proteins work together to create the beautiful and vital structure that is the mitotic spindle.

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http://dx.doi.org/10.1016/j.cub.2014.09.011