Chromokinesins: localization-dependent functions and regulation during cell division

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Abstract

The bipolar spindle is a highly dynamic structure that assembles transiently around the chromosomes and provides the mechanical support and the forces required for chromosome segregation. Spindle assembly and chromosome movements rely on the regulation of microtubule dynamics and a fine balance of forces exerted by various molecular motors. Chromosomes are themselves central players in spindle assembly. They generate a RanGTP gradient that triggers microtubule nucleation and stabilization locally and they interact dynamically with the microtubules through motors targeted to the chromatin. We have previously identified and characterized two of these so-called chromokinesins: Xkid (kinesin 10) and Xklp1 (kinesin 4). More recently, we found that Hklp2/kif15 (kinesin 12) is targeted to the chromosomes through an interaction with Ki-67 in human cells and is therefore a novel chromokinesin. Hklp2 also associates with the microtubules specifically during mitosis, in a TPX2 (targeting protein for Xklp2)-dependent manner. We have shown that Hklp2 participates in spindle pole separation and in the maintenance of spindle bipolarity in metaphase. To better understand the function of Hklp2, we have performed a detailed domain analysis. Interestingly, from its positioning on the chromosome arms, Hklp2 seems to restrict spindle pole separation. In the present review, we summarize the current knowledge of the function and regulation of the different kinocinesins associated with chromosome arms during cell division, including Hklp2 as a novel member of this so-called chromokinesin family.

Introduction

Cell division involves the assembly of a bipolar spindle made of two antiparallel arrays of microtubules that align the chromosomes and segregate them faithfully to the two daughter cells. The assembly of the spindle involves a balance of forces generated by several molecular motors that use the energy derived from ATP hydrolysis to ‘walk’ along the microtubule lattice (reviewed in [1–4]). Mitotic motors perform complementary and sometimes partially redundant functions in microtubule organization and dynamics as well as in chromosome movements. Their functions are in large part dictated by their specific motor properties (e.g. speed, processivity and directionality) [5], but also the temporal and spatial regulation of their activity and localization (e.g. oligomerization, phosphorylation and interactions) [6].

One particular class of kinesins mediates interactions between the chromosome arms and the microtubules participating in spindle assembly and powering chromosome movements. These kinesins were named chromokinesins [7,8]. Until recently chromokinesins belonged to either kinesin-10 or kinesin-4 families. Recently, we showed that the kinesin-12 family member Hklp2 (also named Kif15), localizes specifically to the chromosome arms during mitosis and therefore fulfils the general criteria for chromokinesins [9]. Interestingly, all the chromokinesins also have chromosome-independent localizations to the spindle microtubules with temporal and spatial specificities for each of the members. The existence of three types of chromokinesins raises numerous questions about their function and regulation during cell division. Here, we review the data that have shed some light on the function and regulation of chromokinesins in different organisms during mitosis and meiosis.

Structural features of the chromokinesins

What sets the chromokinesins apart from the other kinesins is their ability to bind to the chromosomes during cell division (Table 1). This localization was first described for Drosophila nod during female meiosis [10–12]. A human kinesin was then found to interact with a specific DNA sequence and named Kid (kinesin interacting with DNA) [13]. Other kinesin-10 family member also associated with the chromosomes was then identified in Xenopus (Xkid) [14,15] and in mouse [16]. The first kinesin-4 family member found to be associated with chromosomes was chicken chromokinesin [7]. Soon after, Xenopus Xklp1 was characterized in the egg-extract system [17,18]. Recently, we showed that Hklp2, a member of the kinesin-12 family, has chromokinesin properties...
Table 1 | Summary of properties of members of the three chromokines in families

<table>
<thead>
<tr>
<th>Family</th>
<th>Name</th>
<th>Species</th>
<th>Chromosome targeting</th>
<th>Motility</th>
<th>Other properties</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinesin-4</td>
<td>Xklp1</td>
<td>Xl</td>
<td>C2H2 zinc finger domain at C-terminus</td>
<td>FL: 0.99 ± 0.07 μm/s in vitro; 0.59 ± 0.07 μm/s in egg extract</td>
<td>Inhibits microtubule dynamic instability; inhibits microtubule growth in antiparallel overlaps</td>
<td>Control of spindle microtubule density and organization, with PRC1 control of anaphase midzone microtubule overlap</td>
</tr>
<tr>
<td>Kif4A/B</td>
<td>Hs</td>
<td>C-terminal ZBZ domain and CR motifs</td>
<td></td>
<td></td>
<td></td>
<td>Spindle bipolarity and metaphase chromosome alignment; chromosome condensation, also non-mitotic functions</td>
</tr>
<tr>
<td>KIF4</td>
<td>Mm</td>
<td></td>
<td></td>
<td>0.034 ± 0.004 μm/s for microtubules; 0.20 ± 0.02 μm/s for axonemes</td>
<td></td>
<td>Chromosome alignment, spindle assembly, cytokinesis, also non-mitotic functions</td>
</tr>
<tr>
<td>Chromokinesin</td>
<td>Gg</td>
<td></td>
<td>Basic-leucine zipper DNA-binding domain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KLP-3B</td>
<td>Dm</td>
<td>In S2 cells, not observed in embryos</td>
<td></td>
<td></td>
<td></td>
<td>Spindle pole separation; establishment/stabilization of central spindle, cytokinesis</td>
</tr>
<tr>
<td>KLP-19</td>
<td>Ce</td>
<td>Localization to mid-bivalent ring during meiosis requires CPC</td>
<td></td>
<td></td>
<td></td>
<td>Polar ejection force, segregation</td>
</tr>
<tr>
<td>Kinesin-10</td>
<td>Xkid</td>
<td>Xl</td>
<td>HhH motif at C-terminus</td>
<td>FL: 0.10 ± 0.01 μm/s in vitro; 0.14 ± 0.01 μm/s in egg extract</td>
<td>Degraded in anaphase by APC/Cdc20 (cell division cycle 20) and APC/Cdh1, involving GXEN motif at C-terminus</td>
<td>Chromosome alignment; meiosis progression</td>
</tr>
<tr>
<td>Kid/Kif22</td>
<td>Hs</td>
<td>Binds DNA through HhH motif at C-terminus</td>
<td></td>
<td>ATP-independent microtubule-binding site; Thr463 phosphorylation by cyclin B Cdc2 down-regulates microtubule affinity</td>
<td>Polar ejection force; chromosome arm orientation and oscillation, spindle size; microtubule bundling</td>
<td></td>
</tr>
<tr>
<td>Kid</td>
<td>Mm</td>
<td></td>
<td></td>
<td>MD: 160 nm/s</td>
<td></td>
<td>Anaphase chromosome compaction in female meiosis I</td>
</tr>
</tbody>
</table>

Abbreviations used: APC, anaphase-promoting complex; Ce, Caenorhabditis elegans; CPC, chromosomal passenger complex; Dm, Drosophila melanogaster; FL, full-length; Gg, Gallus gallus; Hs, Homo sapiens; MD, motor domain; Mm, Mus musculus; n.d., not determined; Xl, Xenopus laevis.
Table 1 | Continued

<table>
<thead>
<tr>
<th>Family</th>
<th>Name</th>
<th>Species</th>
<th>Chromosome targeting</th>
<th>Motility</th>
<th>Other properties</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nod</td>
<td>Dm</td>
<td>Binds DNA through high-mobility domain</td>
<td>Non-motile; microtubule-activated ATPase activity</td>
<td>Microtubule plus-end tracking; stimulates microtubule polymerization</td>
<td>Chromosome arm movements in female meiosis; achiasmate chromosomes positioning</td>
<td></td>
</tr>
<tr>
<td>Kinesin-12</td>
<td>Xklp2</td>
<td>Xi</td>
<td>Not observed</td>
<td>MD: 3.2 ± 1.2 μm/min</td>
<td>Requires TPX2 for microtubule localization</td>
<td>Spindle pole separation</td>
</tr>
<tr>
<td>Hklp2/Kif15</td>
<td>Hs</td>
<td>Through Ki67</td>
<td>Requires TPX2 for microtubule localization</td>
<td>Spindle pole separation; maintenance of metaphase spindle bipolarity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIF12/15</td>
<td>Mm</td>
<td>Not observed</td>
<td></td>
<td>Position spindle poles during prometaphase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KRP180</td>
<td>Dm</td>
<td>Not observed</td>
<td></td>
<td>Establishment and/or maintenance of spindle bipolarity in oocyte</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KLP-54D</td>
<td>Dm</td>
<td>n.d</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>KLP-18</td>
<td>Ce</td>
<td>Not observed</td>
<td></td>
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</tbody>
</table>

[9,19]. Although kinesin-12 family includes orthologues in *Xenopus, Caenorhabditis elegans*, mouse and sea urchin, so far only the human orthologue has been shown to localize to chromosomes [20–23] (Table 1).

All chromokinesins share basic structural features that are common to most kinesins: an N-terminal motor domain, a stalk domain containing predicted coiled-coil regions and a C-terminal tail domain (Figure 1). The presence of the motor domain at the N-terminus is consistent with the plus-end-directed motility shown for several members: Kid [24], Xkid [25] (kinesin-10), mouse KIF4 [26], *Xenopus* Xklp1 (kinesin-4) [27] and *Xenopus* Xklp2 [20] (kinesin-12) (Table 1 and Figure 1). However, Drosophila nod was shown to be non-motile [28]. Although all chromokinesins have predicted coiled-coil regions, these are very different in length. Kinesin-4 members have an extended coiled-coil involved in dimerization [29], whereas kinesin-10 only has a short predicted coiled-coil. By contrast, kinesin-12 has an extended coiled-coil down to a leucine zipper at the very C-terminus essential for dimerization [30] (Figure 1).

The targeting of chromokinesins to the chromosomes is driven by different sequence motifs and interactions [10] (Table 1). An HhH (helix–hairpin–helix) motif present at the C-terminus of kinesin-10 was found to be involved in DNA binding [13,14]. A C-terminal ZBZ (zip/basic/leucine zip) domain and CR (cysteine-rich) motifs in human KIF4A have been shown to be critical for chromatin binding [31]. The kinesin-12 Hklp2 was found to target chromosomes through a sequence in the coiled-coil that does not present any specific motif [19], but interacts with Ki67, a protein that decorates the chromosomes during mitosis and is commonly used as a proliferation marker [9,19,32].

Kinesin-10 and kinesin-4 contain nuclear localization signals in their stalk domain and accordingly they are imported into the nucleus in interphase [14,15,18,33] (Figure 1).

**Chromosome-microtubule interactions mediated by chromokinesins**

Despite the similarities in protein organization and chromosome localization, chromokinesins perform non-redundant functions during cell division, including chromosome alignment and spindle organization, as well as the regulation of microtubule dynamics and chromosome condensation.

Chromokinesins are excellent candidates for powering chromosome movements. Chromosome congression to the metaphase plate involves pulling forces exerted at the kinetochores and pushing forces acting on the chromosome arms that were named ‘polar-ejection forces’ or ‘polar wind’ [34]. Work done in the *Xenopus* egg-extract system showed that the kinesin-10 Xkid is indeed required for metaphase chromosome alignment [14,15]. In human cells, Kid was shown to generate chromosomes oscillations, but, in contrast with its *Xenopus* orthologue, Kid seems to be dispensable for chromosome congression to the metaphase plate [35]. Kinesin-4 family members have also been proposed to play a role in chromosome alignment. However, the role of Xklp1 in microtubule dynamics and organization [18,27,36,37] as well
as the putative role of KIF4A in chromosome condensation [33] suggest that these kinesin-4 members function in multiple processes not directly related to chromosome movements. The functional differences between kinesin-10 and kinesin-4 may in large part be explained by their specific motor properties. Kinesin-4 was found to influence microtubule dynamics freezing both growth and shrinkage in vitro [27]. Consistently, depletion experiments in egg extract showed that the lack of Xklp1 resulted in an increase of microtubule mass in the spindle [37]. Interestingly, the motor properties of the kinesin-10 Xkid and the kinesin-4 Xklp1 determined in vitro on reconstituted chromatin [28] are very different. Xkid is slower than Xklp1, but dominates in the generation of chromosome arm motility due to a more efficient interaction with microtubules. Hklp2 does not play a role in chromosome alignment. Moreover, interfering specifically with Hklp2 targeting to the chromosome by silencing Ki67 has no effect on chromosome alignment but instead generates longer bipolar spindles, suggesting that spindle-pole separation is enhanced. Consistently, these spindles are more resistant to collapse when kinesin-5 is inhibited with monastrol [9]. These results suggest that kinesin-12 at the chromosomes restrains the force is not needed [41]. This bundling activity may help other motors such as kinesin-5 or kinesin-12 to separate the spindle pole. Indeed it was recently shown that microtubule bundling activates Eg5 motility [42].

Similar to kinesin-10, kinesin-12 also localizes to the spindle microtubules. Although kinesin-12 is a cytoplasmic motor in interphase, it only localizes to the microtubules in mitosis. This cell-cycle-specific localization of kinesin-12 depends on TPX2 (targeting protein for Xklp2), a nuclear protein required for the RanGTP-dependent microtubule assembly pathway in mitosis [43,44]. TPX2 was identified as the factor mediating the interaction of Xklp2 C-terminal domain with microtubules [30]. Kinesin-12 and TPX2 may interact directly, at least at some point, but as their localizations differ in time and space, if they interact it may be very transiently [9,45]. A detailed analysis of the dynamics of both proteins on the spindle by FRAP (fluorescence recovery after photobleaching) could give more insights into the timing and strength of this interaction and on its dynamics.

Most functional data obtained for the different orthologues of kinesin-12 indicate a role for this motor in spindle–pole separation. Addition of a C-terminal fragment or antibodies to Xenopus egg extracts suggested a role for Xklp2 in spindle bipolarity [20]; although immunodepletions did not confirm this idea [36,43]. The injection of

Functions of chromokinesins at the spindle microtubules
Apart from their typical chromosome localization, each of the three types of chromokinesins also associates with microtubules in a chromosome-independent way. Kinesin-10 and kinesin-12 localize both to chromosomes and to the microtubules until anaphase, whereas kinesin-4 is exclusively localized to the chromosomes until metaphase and relocates to the spindle midzone during anaphase. The mechanism involved in these chromosome-independent localization to the microtubules is specific for each chromokinesin. Kinesin-10 contains an ATP-independent microtubule-binding site in the stalk [38], suggesting that it can mediate microtubule–microtubule interactions. In addition, the targeting of Kid to the spindle microtubules, but not the chromosomes, depends on its interaction with the protein CHICA [39]. Quite surprisingly, although Kid localizes to the chromosomes in CHICA-silenced cells, chromosomes do not align properly [39]. Further experiments are needed to clarify the reasons for this phenotype and whether it is due to a direct requirement for Kid function at the spindle microtubules or to an effect on other factors altered in the silenced cells. In any case, kinesin-10 has a role in spindle–pole separation that is unrelated to its role in chromosome movements. Indeed, human Kid silencing causes a reduction of spindle size [40,41] that can be rescued by a truncated form of Kid that lacks the DNA-binding domain and localizes exclusively to the spindle microtubules. Therefore Kid has a chromosome-independent function in spindle–pole separation that may rely on a microtubule-bundling activity and that does not require its motor activity, since the generation of an active force is not needed [41]. This bundling activity may help other motors such as kinesin-5 or kinesin-12 to separate the spindle pole. Indeed it was recently shown that microtubule bundling activates Eg5 motility [42].

Figure 1 | Schematic representation of the three chromokinesin families
All share a conserved N-terminal motor domain (white), followed by a stalk consisting of coiled-coil regions of different lengths (light grey). Nuclear localization signals in kinesin-4 and kinesin-10 are indicated in white boxes in the stalk. Black boxes represent regions involved in chromosome targeting: a ZBZ domain and CR motif for kinesin-4, an HHH motif for kinesin-10 and a region that interacts with the chromosomal protein Ki67 for kinesin-12. aa, amino acids.
Phosphorylation by cyclin B–Cdk1 (cyclin-dependent kinase) has been shown to regulate the activity of kinesin-10. This role is crucial as the absence of kinesin-4 causes lagging of chromosomes at the metaphase/anaphase transition when PRC1 loses the Cdk1-dependent phosphorylation, increasing its bundling activity and ability to recruit kinesin-4 [49,52]. Kinesin-4 itself is phosphorylated in mitosis, although the functional relevance of its phosphorylation is unknown [53].

Concluding remarks
The assembly of the spindle and the precise separation of the chromosomes are highly dependent on the collective action of multiple kinesins. In addition, some of these molecular motors are multifunctional. We summarized here the current knowledge on three types of chromokinesins underlying their different functions at the chromosomes and at the microtubules. At the chromosomes, kinesin-10 is responsible for the movements of chromosome arms, kinesin-12 restrains spindle–pole separation and kinesin-4 alters microtubule dynamics controlling the microtubule mass. At the spindle microtubules, kinesin-10 and kinesin-12 contribute to the separation of the spindle poles. Kinesin-4 relocalizes to the midzone microtubules later in mitosis and controls the size of the antiparallel overlap in anaphase. The localization of the different kinesins is controlled at several levels in particular by phosphorylation/dephosphorylation balances and interactions with partners not ubiquitously situated in the spindle. It should be noted that their interaction with different partners at different localizations may also have an influence on their motor biophysical properties [25].

Temporal and spatial regulation of chromokinesins
Cell-cycle-dependent mechanisms prevent the interaction of chromokinesins with microtubules during interphase: kinesin-10 and kinesin-4 are sequestered in the nucleus [8,14,18] and, although kinesin-12 remains in the cytoplasm in interphase, it does not localize to the microtubules (at least in some cells). The mechanism by which this motor is kept from interacting with microtubules in interphase has not yet been elucidated.

After nuclear envelope breakdown, phosphorylation has been shown to regulate the activity of kinesin-10. Phosphorylation by cyclin B–Cdk1 (cyclin-dependent kinase 1) at a site close to the second ATP-independent microtubule-binding site in Kid stalk domain, reduces its affinity for microtubules and therefore controls the distribution of this motor between the microtubules and the chromosomes [38,51].

The ‘activation’ of kinesin-12 occurs after nuclear envelope breakdown in a TPX2-dependent way. Indeed TPX2 is required for Hklp2 localization to both spindle microtubules and chromosomes, although the exact mechanism is still unknown [9].

After metaphase, each chromokinesin changes its localization and/or function. Hklp2 becomes more diffuse and appears to lose its association with the chromosomes [9]. This change may reflect a change in phosphorylation state, as the protein stability is not affected. Additional work is required to address this possibility. The kinesin-10 Kid is degraded in anaphase, but it seems to still associate with the separating chromosomes in somatic cells. This suggests that some mechanisms must ensure its inactivation to allow chromosome segregation in early anaphase. This may be related to its dephosphorylation, although this seems contradictory to the idea that the unphosphorylated form has more affinity to microtubules [51]. In late anaphase, studies in mouse embryos and human cells also showed that Kid has a role in the compaction of the chromosomes during the formation of the nuclear envelope in the daughter cells [51].

The redistribution of kinesin-4 from the chromosomes to the microtubules during late anaphase depends on PRC1 [48]. This is also controlled by a transition in phosphorylation state at the metaphase/anaphase transition when PRC1 loses the Cdk1-dependent phosphorylation, increasing its bundling activity and ability to recruit kinesin-4 [49,52]. Kinesin-4 itself is phosphorylated in mitosis, although the functional relevance of its phosphorylation is unknown [53].
The concept of multiple functions dependent on localization is probably common to many motors, as was already shown to be the case for Eg5 [54]. The combination of studies on the regulation of chromokinesins in vitro together with the use of in vitro systems to obtain detailed biophysical data should provide a deeper understanding of the different mechanisms of action of the chromokinesins and their possible multifunctionality in the spindle.

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References


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