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MAPping kinetochore MAP function required for stabilizing microtubule attachments to chromosomes during metaphase

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Abbreviations: MT(s), Microtubule(s); kMT, kinetochore-microtubule; MAP(s), Microtubule-associated proteins; WTH, Winged-turn-helix; CH, Calponin-homology

Key words: Microtubules, Kinetochores, Mitosis, Chromosomes, MAPs, Ndc80

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Abstract

In mitosis, faithful chromosome segregation is orchestrated by the dynamic interactions between the spindle microtubules (MTs) emanating from the opposite poles and the kinetochores of chromosomes. However, the precise mechanism that coordinates the coupling of kinetochore components to dynamic MTs has been a long-standing question. Microtubule (MT) associated proteins (MAPs) regulate MT nucleation, dynamics, MT-mediated transport and MT cross-linking in cells. Especially during mitosis, MAPs play an essential role not only in determining the spindle length, position and orientation but also in facilitating robust kinetochore-microtubule (kMT) attachments by linking the kinetochores to spindle MTs efficiently. MT-stability imparted by the MAPs is critical to ensure accurate chromosome segregation. This review primarily focuses on the specific function of **non-motor kinetochore MAPs**, their recruitment to kinetochores and their MT-binding properties. We also attempt to synthesize and strengthen our understanding of how these MAPs work in coordination with the kinetochore-bound Ndc80 complex (the key component of the MT-binding interface in metaphase and anaphase) to establish stable kMT attachments and control accurate chromosome segregation during mitosis.

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Introduction

1. Outer kinetochores at a glance - structural and functional components

Kinetochore is a complex multi-protein structure that assembles at the centromere region of chromosomes. Kinetochores connect dynamic mitotic spindle microtubules to sister chromatids to drive equational partitioning of genetic material between the two daughter cells during mitosis (Alushin and Nogales, 2011; Cheeseman and Desai, 2008). Failure or anomalies in this attachment process arrest or delay the mitotic exit via the activation of spindle assembly checkpoint (SAC) - a fail-safe surveillance system that synchronizes attachment of chromosomes to the spindles with the cell cycle progression (Bakhoum and Compton, 2012; Foley and Kapoor, 2013). Amongst the multitude of proteins that populate mitotic kinetochores, an evolutionary conserved network of protein complexes: the KMN network (Knl1-Mis12-Ndc80) functions as the lead conductor in mediating the robust attachment of kinetochores to the spindle microtubules (MTs) (Cheeseman and Desai, 2008; Varma and Salmon, 2012). Within the KMN network, Ndc80, a heterotetrameric dumbbell-shaped complex comprising of four subunits - Hec1, Nuf2, Spc24 and Spc25, contributes largely to the MT-binding function of the kinetochore (Umbreit et al., 2012; Wei et al., 2007); with Knl1 following the suit as the second but less distinctive facilitator (Espeut et al., 2012; Ghongane et al., 2014). The dimeric Hec1/Nuf2 has been shown to bind to MTs even in the absence of Spc24/25. This is attributed to the characteristic folding of the Hec1/Nuf2 N-terminal globular heads into two Calponin-homology (CH) domains (Ciferri et al., 2008; DeLuca et al., 2006; DeLuca and Musacchio, 2012; Wei et al., 2007). The CH domain is a 100 aa tetra-helical structural motif that is commonly found in proteins that bind to cytoskeleton (Korenbaum and Rivero, 2002). In Fimbrin or α-Actinin, several CH-domains are arranged in tandem to promote high-affinity actin binding unlike a single CH-domain in EB1 protein that mediates attachment to

MTs (Gimona et al., 2002). In addition to the CH-domain, an intrinsically unstructured, highly positively charged ~80 aa N-terminal tail within the Hec1 protein was also required for high affinity MT-binding (Alushin et al., 2010; Cheeseman et al., 2006; DeLuca et al., 2006; Guimaraes et al., 2008; Zaytsev et al., 2015). However, the involvement of the Ndc80 tail in MT attachments is variable across species. While in mammalian cells, deletion of the N-terminal tail did destabilize kinetochore-microtubule (kMT) attachments, no obvious perturbation in chromosome segregation was observed in worms or budding yeast (Cheerambathur et al., 2017; Tooley and Stukenberg, 2011).

Notwithstanding the fact that the Ndc80 complex of the KMN network is largely demonstrated as the primary contributor to the MT-binding function, Knl1/Blinkin/AF15q14 has also been shown to function as a conserved MT-binding kinetochore protein that is critical for effective MT attachment; however, its role in this context still remains unclear (Cheeseman and Desai, 2008; Varma and Salmon, 2012).

2. Looping-in on the (Ndc80) loop – how much do we know?

It has been observed that the depletion of Ndc80 could not completely abolish kMT attachment in some experimental systems, even though the attachments were rudimentary and were not able to sustain chromosomal congression and eventual segregation (Ciferri et al., 2007). This result is suggestive of the idea that additional MT-binding proteins at the kinetochores must function either independently or in conjunction with the Ndc80 complex to orchestrate efficient contact with the kMTs. The structural analysis of Ndc80 revealed that the entire central part of the complex is composed of a coiled-coil structure with a kink/loop at approximately 16 nm from the Hec1/Nuf2 dimer head (Wang et al., 2008). While the sequence of this loop is not strictly conserved, its

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position within the coiled region is relatively fixed. The loop primarily imparts some degree of flexibility by introducing a break in the otherwise long and rigid coiled coil structure of Ndc80 (Nilsson, 2012). Although the loop does not contribute to the MT-binding directly and is separated quite a distance from the MT-binding activity residing within the globular Hec1/Nuf2 heads, it is interesting that there has been a recent upsurge in studying the unique role of this internal loop region in establishing end-on attachments with MTs for accurate chromosomal segregation (Nilsson, 2012; Tang and Toda, 2013; Zhang et al., 2012). Thus, it is imperative to understand the intricacies of how the loop domain of Ndc80 participates in modulating the spindle MTs during cell division.

There is a plethora of evidence available from yeast to humans that the loop domain of Ndc80 functions as a docking hub for several proteins that can bind to MTs at the kinetochores (Hsu and Toda, 2011; Maure et al., 2011; Tang and Toda, 2013; Varma et al., 2012; Zhang et al., 2012). To name a few: the Dam1/DASH complex in budding yeast, Dis1 in fission yeast, the Ska complex and Cdt1 in humans are all important components recruited to kinetochores with the assistance of the loop domain. A common theme that emerges from these findings is that the loop domain could potentially function to recruit several MT-binding proteins at the kinetochores that can optimally augment the MT-binding ability of the Ndc80 complex. Thus, the loop region of Ndc80 can be compared to an integrating, multichannel circuit where additional "loops" feed in simultaneously or independently of each other to regulate or amplify the output. While, some of the proteins that dock on to the Ndc80 loop region have been identified and studied in great detail, additional ones that can potentially feed into the Ndc80 loop to possibly provide temporal control of kMT attachments in a stage-specific manner during mitosis are yet being discovered. Whether via loop or without it, it is interesting that all these proteins absolutely need Ndc80 complex for

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their localization to the kinetochores and their role is to ultimately serve Ndc80 especially in augmenting its MT-binding function.

3. *A functional overview of mitotic MAPs required for chromosome alignment and segregation*

During mitosis, an entire array of molecular machinery i.e. the mitotic spindle is built from MTs, which plays an essential role throughout the whole process of chromosome capture, congression, and segregation followed by accomplishment of successful cytokinesis (Desai and Mitchison, 1997). The ability of MTs to perform a plethora of diverse functions originate from their intrinsic dynamic behavior that is regulated and coordinated by a dedicated family of proteins referred to as microtubule associated proteins (MAPs) (Akhmanova and Steinmetz, 2008). By virtue of their ability to regulate the MT turnover, stability and dynamics, MAPs are involved in a wide variety of processes such as guiding the MTs towards specific cellular locations, cross-linking the MTs, and facilitating interaction of other proteins with the MTs. Mitotic MAPs can be broadly categorized into 4 types based on their function: (1) MAPs that promote and stabilize MT polymerization, (2) MAPs which lead to MT destabilization or severing, (3) MAPs functioning as spindle MT cross-linkers, and (4) MAPs that participate in kinetochore motility. These proteins may also function as motor or non-motor MAPs based on their structural and enzymatic properties reviewed by Petry S (Petry, 2016).

(a) Motor MAPs

MAPs serving as motors are mechano-enzymes that utilize the energy from ATP hydrolysis to physically walk along the length of MTs. The most widely and exhaustively studied motor MAPs are the mitotic kinesin superfamily including the MT-depolymerizing kinesin 13s (e.g., Kif2s, MCAK, and Klp10A) and the kinesin 8s (e.g., Kif3 and Kif18A), both being MT plus-end

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directed motors (Wordeman, 2010). In contrast, the kinesin 14s (e.g. Kar3, Ncd, KlpA, HSET and Xctk2) (Daire and Pous, 2011; Furuta et al., 2008; Wordeman, 2010) are MT minus-end directed motors.

The other extremely well studied motor MAPs are the kinetochore-associated MT motors including cytoplasmic dynein and the kinesin-7 subfamily member, centromeric protein E (CENP-E), that move towards MT minus-ends and plus-ends, respectively (Kapoor et al., 2006; Kardon and Vale, 2009; Kim et al., 2010). In addition to the diverse array of mitotic functions carried out by cytoplasmic dynein, like maintaining normal mitotic spindle architecture and chromosome alignment, it is also required for generating robust end-on kMT attachments in metaphase (Varma et al., 2008; Yang et al., 2007). CENP-E has also been reported to stably associate with both the assembling and disassembling MT tips and thus plays an active role in linking kinetochores and dynamic MT tips after the establishment of end-on kMT attachment (Gudimchuk et al., 2013).

Yet another class of MT-plus-end directed motor MAPs that associate with the chromosome arm rather than the kinetochores are the kinesin-10 family members including the chromokinesin, Kid, which are proposed to stabilize the kMT attachments (Drpic et al., 2015). In summary, the motor MAPs use MTs as rail-tracks to move the kinetochores in '+', anterograde and '-', retrograde directions as required during chromosome alignment and segregation. Since the primary goal of this review is to highlight the function of non-motor MAPs required for stabilizing kMT attachments during metaphase and anaphase, we refrain from an extensive discussion on the function of motor MAPs during mitosis, about which several other reviews have already been published (Maiato et al., 2017; Welburn, 2013).

(b) + TIPs

Unlike the motor MAPs that bind throughout the surface of MT lattice, other MAPs popularly called as +TIPs, distinctively associate with the plus-ends of MTs and are inherently predicted to be required for stabilizing the attachments between MT plus-ends and kinetochores in metaphase (Ferreira et al., 2014; Tamura and Draviam, 2012). The first reported plus-end tracking protein was the 170 kDa cytoplasmic linker protein, CLIP-170. Since its discovery, more than 20 different +TIP families have been identified. Some +TIPs including EB1 (End-binding 1), chTOG (known as XMAP215 in *xenopus* and CKAP5 in humans), CLIP-170, CLIP-associated proteins (CLASPs) and their homologs promote polymerization of MT plus-ends; while others including kinesin-13/MCAK depolymerize MTs at the plus-ends (Akhmanova and Steinmetz, 2008; Akhmanova and Steinmetz, 2010). EB1 and chTOG are unique +TIPs, since they can autonomously accumulate at the MT plus-ends without the assistance of other MAPs (Akhmanova and Steinmetz, 2008; Akhmanova and Steinmetz, 2010). Since, EB1 of EB family proteins can recruit most of the other +TIPs and several other MAPs to the plus-ends of MTs through direct protein-protein interactions, it is often considered as “the master +TIP”.

EB family proteins consist of four functional regions; the N-terminal calponin homology (CH) domain which is typically found in actin-binding and signaling proteins, and is responsible for MT-binding (Akhmanova and Steinmetz, 2008), the central coiled-coil domain for homo-dimerization, the EB homology (EBH) domain and a disordered acidic tail encompassing a C-terminal EEY/F motif (Matsuo et al., 2016). The EBH domain specifically binds to a variety of other +TIPs that contain the SxIP (x=any amino acid) motif, including CLASPs, adenomatous polyposis coli (APC), microtubule-actin crosslinking factor (MACF) and MCAK. The short linear SxIP motif serves as a general MT tip localization signal for these proteins (Honnappa et al., 2009). The C-terminal EEY/F motifs of EB family proteins guide certain other MAPs including CLIP-

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170 and p150^{glued} to MT-plus ends by binding to cytoskeleton-associated protein-glycine rich (CAP-Gly) domains with a well-conserved GKNDG sequence motif within these proteins (Li et al., 2002; Saito et al., 2004; Weisbrich et al., 2007). The LxxPTPh (where x=any amino acid and h=any hydrophobic amino acid) motif was recently identified as a third EB-binding motif that enabled major +TIPs including yeast Kar9 and human TACC1 to interact with the EB proteins at MT ends (Kumar et al., 2017). However, the evidence that EB1 or the other factors that it recruits to MT plus-ends, is required for the stabilization of kMT attachments, is scanty. Further, since our primary focus is on non-motor kinetochore MAPs required for stable kMT attachments, an elaborate discussion on EB1 and its binding partners is beyond the scope of this review.

In contrast, another class of + TIPs that have been clearly demonstrated to be required for stabilizing kMT attachments are the XMAP215 family of proteins. These proteins localize to the extreme MT plus-ends, in contrast to the EB1 family that bind to an extended region, and in addition localize also to mitotic kinetochores (Al-Bassam and Chang, 2011). The functions of the XMAP215 family members will be discussed in detail in the succeeding section on non-motor kinetochore MAPs that are required for stable kMT attachments during mitosis.

4. Kinetochore MAPs required for robust kMT attachment during metaphase and anaphase

Kinetochores establish stable association with dynamic MT ends, which undergo constant assembly and disassembly. In this section, we will comprehensively analyze the characteristics of several proteins that are recruited to kinetochores by the Ndc80 complex and contribute to the robust kMT attachment during metaphase and anaphase.

(a) Cdt1

Cdt1 (Cdc10-dependent transcript 1) protein primarily functions in the licensing of the origins during DNA replication at the G1 phase of the cell cycle. Therein, Cdt1 serves as a "courier" that delivers and loads soluble MCM (Minichromosome Maintenance) molecules to the DNA-bound ORC (Origin Recognition Complex)/Cdc6 (Cell Division Cycle 6) complex, marking the completion of the presumptive pre-RC (pre-replication complex) (Pozo and Cook, 2016). Interestingly, our previously published work had demonstrated a second essential role of Cdt1 during the M-phase of the cell cycle as well; wherein Cdt1 was shown to localize to kinetochores through an interaction with the Hec1 component of the Ndc80 complex (Varma et al., 2012). Two-hybrid screening in yeast and *in vivo* GST pull down assay using asynchronous HeLa cell lysates confirmed Cdt1-Hec1 direct interaction (Varma et al., 2012), which was further substantiated in our recent study using purified proteins in a blot overlay assay (Agarwal et al., 2018).

High-resolution microscopy data indicated that the average separation, Delta, between the two ends of the Ndc80 complex was considerably lower in Cdt1-depleted cells as compared to the control metaphase cells where kinetochores attain a full MT complement (Varma et al., 2012). Although this data suggested that Cdt1 binding to the Ndc80 loop domain provided an additional attachment site, which was essential for an extended Ndc80 configuration and stable kMT attachment, the exact mechanism of how Cdt1 was functioning in generating the observed phenotypes was still not delineated. Our recent study explicitly uncovers the mechanistic details of how Cdt1 contributes to the stabilization of kMT attachments (Agarwal et al., 2018). *In vitro* and *in vivo* studies show that Cdt1 directly binds to MTs through an extended segment comprising of its middle and C-terminal regions (**Fig. 1A1, Fig. 2A**). This segment, while largely being unstructured, consists of two Winged-turn-helix (WTH) domains, one in the middle and the other at the C-terminus, that is predicted to be involved in MT-binding. The N-terminal region of Cdt1

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on the other hand, binds to the Ndc80 complex (Agarwal et al., 2018; Varma et al., 2012) thus, serving to bridge the loop domain of the complex to spindle MTs. The 2nd MT-binding interface mediated by the loop domain and Cdt1, likely contributes to more robust kMT attachments formed by the Ndc80 complex (Fig. 1A2, Fig. 2A). Similar to the Ndc80 complex, Aurora B kinase negatively regulates the MT-binding activity of Cdt1 (**Fig. 1A2**). *In vivo* functional rescue experiments using Aurora B phosphomimetic mutants of Cdt1 generates severely defective KMT attachments and impaired normal mitotic progression (Agarwal et al., 2018), mirroring the Cdt1 loss-of-function phenotype in mitotic cells (Varma et al., 2012).

The Winged-turn-helix (WTH) domain, the predicted MT-binding domain of Cdt1, is one of the core components of the transcription machinery, and has the ability to bind DNA and to mediate protein-protein interactions (Gajiwala and Burley, 2000; Teichmann et al., 2012). The canonical WTH domain (~110 amino acids) typically has a compact α/β structure consisting of two wings or loops (W1, W2), three α helices (H1, H2, H3) and three β-sheets (S1, S2, S3) arranged as H1-S1-H2-H3-S2-W1-S3-W2 (Gajiwala and Burley, 2000). Kinetochore-localized Ska1 protein was the first instance where the WTH domain was shown to function as a MT-binding domain (Abad et al., 2014; Jeyaprakash et al., 2012). The WTH domain of Ska1 differs from that of a canonical WTH domain, in that it has two extra elements [module I comprising of two α helices (α1 and α2) at the N-terminal and module II of three α helices (α4, α5 and α6) in the middle] in addition to the core elements (Abad et al., 2014). The WTH domains of Cdt1 on the other hand is more similar to canonical WTH domains except that the C-terminal WTH domain has an extra α helix in addition to the core elements (Agarwal et al., 2018). It is interesting that DNA binding motifs could be shared and used as MT-binding motifs because both the DNA and MTs are negatively charged moieties and thus, are recognized by proteins via electrostatic interactions.

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These domains thus represent a contemporary class of unconventional MAP-motifs that can impart MT-binding function to MAPs.

The present understanding is that in the G1 phase, Cdt1 associates only indirectly with the nuclear chromatin through the origin recognition complex (ORC) and coordinates with Cdc6 to load the MCM2-7 helicase complex onto the chromatin to license DNA replication (Symeonidou et al., 2012; Tsakraklides and Bell, 2010). However, one study has shown that anti-Cdt1 antibody efficiently immunoprecipitated DNA sequences, thus suggesting the possibility of a direct interaction between Cdt1 and DNA (Sugimoto et al., 2011). At this point, we can only surmise that this direct interaction is likely mediated by the WTH domains of Cdt1; however, without any experimental validation, the finer details of Cdt1 DNA-binding still remain unclear. Additionally, since Ska is an outer kinetochore protein that has not been reported to localize to the nucleus in interphase, unlike Cdt1; it is expected that the WTH domain of Ska exclusively binds MTs. To the best of our knowledge, there is also no available evidence of DNA-binding proteins that are also capable of binding to the MTs. In summary, the evidence is currently scanty that the "conventional" DNA-binding WTH and the "unconventional" MT-binding WTH domains can be used by the same protein to bind to both DNA and MTs interchangeably.

Till date, hundreds of MAPs have been discovered and many novel ones continue to emerge. Recently, an online MAP predictor algorithm, "MAPanalyzer" was developed to compile the existing and predict the potential MAPs (Zhou et al., 2015). To address whether it deems suitable to claim Cdt1 as a bona-fide MAP, we subjected the sequence of human Cdt1 through this algorithm employing a moderately sensitive filter (>90%). This analysis suggests that Cdt1 can potentially function as a MAP (http://systbio.cau.edu.cn/mappred/pq.php?id=20180613052444); corroborating with our recent finding demonstrating Cdt1 as a novel MT-binding protein (Agarwal

et al., 2018). Additionally, conventional MAPs like Tau contain an exorbitant amount of proline residues or proline rich regions, which are implicated in MT binding (He et al., 2009). On an average, the proline content of any human protein is 6.3% (Morgan and Rubenstein, 2013), and interestingly proline constitutes 10.8% of the human Cdt1; in fact, 15-109 amino acid region of Cdt1 is 24.2% proline. Moreover, MAPs are overall or partially disordered, but they attain proper conformation and stability when they bind to MTs. In accordance with this line of thought, the N-terminal 93 amino acids of Cdt1 are shown to be at least ~35% intrinsically disordered, and the N-terminal region (1-160) exhibited limited secondary structure elements in the absence of other binding partners (Agarwal et al., 2018). Thus, based on these afore-mentioned criteria, Cdt1 could be classified as a novel kinetochore MAP.

(b) The Ska complex

The heterotrimeric spindle and kinetochore-associated (Ska) complex, formed by the association of three subunits (Ska1, Ska2, and Ska3/Rama1), is enriched at the kinetochores of aligned chromosomes and has been shown to bind to MTs (**Fig. 1B2, Fig. 2A**) (Gaitanos et al., 2009; Raaijmakers et al., 2009; Schmidt et al., 2012; Welburn et al., 2009). The Ska complex has also been implicated in SAC silencing (Daum et al., 2009; Hanisch et al., 2006) by recruiting the PP1 phosphatase, thereby facilitating mitotic exit (Sivakumar et al., 2014; Sivakumar et al., 2016). The core of the Ska complex is W-shaped composed of dimers of coiled-coils generated by the intertwined interactions between Ska1, Ska2, and Ska3 (Jeyaprakash et al., 2012). Each Ska subunit has its unique and specific contribution within the complex for it to function as a complete unit. While Ska1 mediates interaction between Ska2 and Ska3, and its C-terminus serves as the main MT-binding component of the complex, Ska2 and Ska3 constitute the scaffold of the dimerization interface (Jeyaprakash et al., 2012). The C-terminus of Ska3 has also been shown to

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provide additional MT-binding ability (Gaitanos et al., 2009). The C-terminal domains of Ska1 and Ska3 have been shown to protrude symmetrically on both sides of the W-shaped central core to contact the MTs, but that is possible only when they are connected to the central core (Jeyaprakash et al., 2012). These findings clearly suggest that the central core of Ska1 and Ska3 by itself is inert for MT binding, but it serves to position the peripheral MT-binding domains of the complex in an appropriate geometry.

Although depleting the Ska subunits does not affect the localization of Ndc80 or any other KMN components, it only impairs kMT attachments; the converse is not true, i.e. Ska complex absolutely requires Ndc80 for its localization at the kinetochores (**Fig. 1B1, Fig. 2A**) (Gaitanos et al., 2009; Welburn et al., 2009). A recent study shows that the Ska complex directly binds to Ndc80 complex and increases the affinity of the Ndc80 complex for MTs, thus enabling the formation of stable kMT attachments (Helgeson et al., 2018). As expected, both Aurora B kinase phosphomimetic Hec1 N-terminal tail mutant and a mutant Ndc80 complex lacking the entire unstructured N-terminal 80-aa Hec1 tail, formed weak attachments to the MTs compared to the wild-type Ndc80 complex. However, upon addition of the free Ska complex, the stability of MT attachments was not just regained, but was also reinforced by 5-fold. This also suggests that the Ska complex-mediated strengthening of MT attachments is independent of the Hec1 N-terminal tail (Helgeson et al., 2018).

Jeyaprakash *et al*, eloquently elaborates on the interesting parallels between the overall design and arrangement of these two complexes-Ska and Ndc80. The central core of both is composed of coiled-coil regions decorated with peripheral domains on either end. While the Ndc80 coiled-coil has MT-binding domains at one-end and kinetochore-binding domains at the other; the Ska complex is far more symmetrical in its architecture in the sense that it has MT-binding domains

on each end. This symmetrical nature of the Ska complex provides it with the unique and well-suited ability to contact a MT at two sites simultaneously. This flexibility is typical of many MT-binding proteins that allows for the unbiased diffusional motility (Cooper and Wordeman, 2009).

In general, MT tracking proteins are primarily considered for their association with the MT lattice or the polymerizing ends; but the depolymerizing ends, also represent a potential site of interaction. The Ska complex has been shown to associate with not only depolymerizing MT ends, but also equally efficiently with the straight MT lattice and curved MT protofilaments. On the other hand, the human Ndc80 complex binds only to the MT lattice, and lacks tracking activity of polymerizing or depolymerizing MT ends (Schmidt et al., 2012). Interestingly, however, the yeast Ndc80 complex has been shown to be essential for the formation of load-bearing attachments during both MT assembly and disassembly (Powers et al., 2009).

Analysis of Ska1 sequence across various organisms shows that like EB1 protein, Ska1 also contains a nearly perfect and conserved "*microtubule tip localization signal*" (MtLS)-SxIP motif (Honnappa et al., 2009), "SHLP" at its N terminus (Thomas et al., 2016). Interestingly, analysis of Cdt1 sequence did not reveal presence of any such SxIP or related motif(s). The C-terminal coiled-coil domain of EB1 has been shown to interact with numerous +TIPs through their conserved SxIP motifs. Interestingly, Ska1 recruitment to the plus ends, and its interaction with EB1 was shown to involve the same C-terminal region of EB1 that interacts with the SxIP motifs of other +TIPs (**Fig. 1B2**) (Thomas et al., 2016). Several plus-end tip-tracking proteins that associate with elongating MTs through the end-binding (EB) family of proteins can simply be considered as "hitchhiker" molecules, and not as "autonomous" tip trackers. However, *in vitro* analyses indicate that the Ska1 complex is capable of tracking polymerizing MTs independent of EB1 (Monda et al., 2017). Monda *et al* observed bright fluorescence of GFP-Ska1 complex at the

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growing MT ends (**Fig. 1B2**). In addition to that, when MT depolymerization was induced by the removal of soluble tubulin, the Ska1 complex also showed enrichment at the shortening MT ends (Monda et al., 2017). Thus, the Ska complex exhibits the remarkable ability to remain associated with both polymerizing and depolymerizing MT plus-ends, a property that has so far been reported only in one other human kinetochore MAP, the CENP-E motor (Gudimchuk et al., 2013).

Even though both Cdt1 (Agarwal et al., 2018) and Ska (Schmidt et al., 2012) demonstrated lower speed, bi-directional diffusive motion on MTs; this characteristic was substantially different from the directed, high-speed motility exhibited by motor MAPs such as kinesins. These observations are both indicative and supportive of the fact that kinetochore proteins like Cdt1 and Ska resemble conventional MAPs to some, if not to the full, extent. It is fascinating that even though Cdt1 and Ska do not share any substantial sequence or structural similarities, they both have functional similarities with respect to binding to the Ndc80 loop for kinetochore recruitment and interacting with MTs. It is still unclear which regions these proteins employ to dock on to the loop, and whether one is required for the assembly of other. For the recruitment of the Ska complex to the kinetochores, though Ndc80 has been recognized as a receptor (Chan et al., 2012; Gaitanos et al., 2009; Raaijmakers et al., 2009; Welburn et al., 2009); the specific involvement of the loop domain within the Ndc80 complex has been clearly demonstrated (Zhang et al., 2012; Zhang et al., 2017). In support of the above findings, Cdk1-mediated phosphorylation of Ska3 has been shown to promote its association with the Ndc80 loop domain. Concomitantly, the Ska3 mutants deficient in Cdk1 phosphorylation failed to localize to kinetochores, but retained their MT localization (Zhang et al., 2017). Contrary to these studies, fairly recently, an alternate mechanism for Ska recruitment to kinetochores has also been demonstrated i.e. via the N-terminal unstructured tail of Ndc80 (Janczyk et al., 2017). Another study demonstrated that the direct interaction of the

Ska complex (Ska1 subunit) with the MT plus-end binding EB1 protein was responsible for the localization of Ska1 to the kMT interface (Thomas et al., 2016). At this point, it suffices to say that despite several studies, the architecture of the interaction interface between the Ndc80 and Ska complexes, and how they assemble at the kMT interface remains poorly defined. The most recent contribution in this connection is provided by a study from Helgeson *et al*. Employing cross-linking and mass spectrometry with Ska complex, Ndc80 complex, and taxol-stabilized MTs, Ska3 unstructured C terminal region (residues 102–412) was found to cross-link robustly with the Ndc80 complex in the regions that are predicted to form coiled-coils (Helgeson et al., 2018). Further mapping revealed that few interactions were also distributed among the 4 subunits of the Ndc80 complex i.e. some cross-links were observed with the CH domains of Hec1 and Nuf2 or the RWD domains of Spc24 and Spc25 (Helgeson et al., 2018). In summary, the data from these studies suggest that multiple Ndc80-dependent mechanisms may function in parallel, to recruit Ska to the kinetochores.

Based on these characteristics, qualifying these two above-mentioned kinetochore-associated proteins, Cdt1 and Ska complex as conventional MAPs seems less compelling and warrants further and thorough investigation. Albeit, these kinetochore proteins may not be quintessential MAPs, they can safely be categorized as multifunctional MAPs; since they share few but not all the characteristics, which are hallmarks of conventional MAPs. It is also presumed that the multifunctional MAPs may not be able to perform their MT-related function effectively by themselves/alone, and may depend on some other MAP(s) or protein(s) or some sort of direct or feed-back regulation like phosphorylation for modulating their affinity to bind to MTs. Indeed, Cdt1 and Ska both conform to this hypothesis as well where phosphorylation by Aurora B kinase has been shown to not only influence their interaction with MTs (Abad et al., 2014; Agarwal et

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al., 2018) but also affects their localization to kinetochores as shown at least for the Ska complex (Chan et al., 2012).

Another interesting question worth deliberation is whether the kinetochore binding ability of these MAPs influences their ability to interact with a specific region of spindle MTs such as the plus-ends. Interestingly, it has been shown that the Ska complex is able is localize to kinetochores only after kMT attachments have been formed (Chan et al., 2012; Hanisch et al., 2006). However, no such correlation has been discerned yet for Cdt1, since Cdt1 was able to localize normally to kinetochores (in both normal and nocodazole-treated cells) as well as to the spindle MTs (Agarwal et al., 2018; Varma et al., 2012). In fact, the staining of Cdt1 was more evident at the spindle poles as compared to the rest of the spindle, which could be due to the high density of MTs at that location. Similar to Cdt1, myc-tagged Ska1 has been shown to localize to kinetochores in addition to its ability to partly co-localize with spindle MTs and also the spindle poles in mitotic cells (Hanisch et al., 2006). These observations suggest that the localization of Ska or Cdt1 to kinetochores is not required for their MT-binding. Since the localization of Cdt1 to kinetochores and to MTs are seemingly independent events, binding of Cdt1 to one site does not impede its localization to the other site. In fact, we feel that this unique ability of Cdt1 to localize to both the kinetochores and spindle MTs might be crucial for its function in robust kMT attachment formation.

(c) Dam1/DASH complex

In budding yeast *Saccharomyces cerevisiae*, the heterodecameric Dam1 or DASH complex couples kinetochores to MT ends (Tanaka and Desai, 2008). Interestingly, while Dam1/DASH complex is essential for survival in budding yeast, it is dispensable in fission yeast (Joglekar et al., 2010). This ten-protein complex contains Dam1p, the main MT binding component (Hofmann et

al., 1998) along with the nine other essential proteins; Duo1p, Dad1p, Dad2p, Spc19p, Spc34p, Ask1p, Dad3p, Dad4p and Hsk3p (McAinsh et al., 2003). Mutations in DASH subunits resulted in unstable or broken spindles that consequently led to weakened kMT attachments (Cheeseman et al., 2001). Purified Dam1/DASH complexes have been shown to coalesce into stable ring-like structures or open spirals around MTs *in vitro*, which can slide passively along the MT lattice (**Fig. 1F2, Fig. 2B**) (Westermann et al., 2005; Westermann et al., 2006). This unique property of Dam1/DASH complex to form rings around the MT lattice and its preferential binding to GTP-tubulin makes it stand apart from a number of other MAPs studied so far. The Dam1 complex, but not the Ndc80 complex, was shown to possess an intrinsic plus end–tracking ability. However, Dam1 was shown to mediate continuous Ndc80 tip association with dynamic MTs, suggesting that in yeast, Dam1 complexes are the primary source of kinetochore plus end–tracking activity, whereas Ndc80 complexes structurally bridge MT ends with chromosomes (Lampert et al., 2010). Moreover, the Ndc80 complex is absolutely essential for the recruitment and assembly of Dam1 complex onto the kinetochores (**Fig. 1F1, Fig. 2B**) (Janke et al., 2001). The Ndc80 loop region has been shown to be essential for Ndc80-Dam1 interaction and kinetochore loading of the Dam1 complex (Maure et al., 2011). Evidence for an interaction between these two complexes has been provided by localization and two-hybrid studies (Joglekar et al., 2009; Shang et al., 2003). Phosphorylation of Dam1 at Ser20 by the yeast Aurora B kinase homologue Ipl1 has been shown to reduce its affinity for the MT lattice (Gestaut et al., 2008). Further, phosphorylation of the components of Dam complex (Dam1p, Ask1p, and Spc34p) has been shown to negatively impact its interaction with the Ndc80 complex (Shang et al., 2003; Tien et al., 2010); however, which phosphoprotein component of the Dam complex contributes to the disruption of interaction is still unclear. Recently, an interesting study by Kim *et al* has shown that the Ndc80 complex can

simultaneously bind and bridge two Dam1 complex rings not one (Kim et al., 2017), as was presumed before (Joglekar et al., 2009; Tanaka et al., 2007). Each component within this tripartite interaction was shown to be regulated by Aurora B kinase. Further, mutating any one of the Dam1-Ndc80 interacting regions to generate defective Ndc80 rods that could bind to only one Dam ring *in vitro* resulted in erroneous chromosomal segregation and weakened kMT attachments *in vivo* (Kim et al., 2017). The study extended to show that the specific distance between the two rings, bridged by the extended Ndc80 complex is also crucial for accurate cell division (Kim et al., 2017). Interestingly, based on the functional similarities and dependence on Ndc80 complex for kinetochore recruitment, the Ska complex has been pitted as a functional equivalent of the Dam1/DASH complex in humans.

(d) XMAP215 family

The XMAP215 family proteins were first identified as MAPs that regulate the dynamic properties of MTs (Mack and Compton, 2001). This is the only known family of kinetochore MAPs whose members have been investigated from yeast to mammals. The XMAP215 family orthologues include colonic hepatic tumor over-expressed gene (chTOG) in humans (Charrasse et al., 1998), XMAP215 (most studied member of the family) in *Xenopus*, Stu2 in budding yeast, Dis1/Alp14 in fission yeast, Zyg9 in worms, and Minispindles (MSPS) in *Drosophila* (Al-Bassam and Chang, 2011). These proteins are localized at MT plus-ends, MT-organizing centers, kinetochores and along MT lattices. A characteristic feature of this family of proteins is an N-terminal repeating structure and a more or less conserved C-terminal non-repeat domain (Hsu and Toda, 2011; Lee et al., 2001; Li et al., 2011; Spittle et al., 2000; van der Vaart et al., 2011). The N-terminal repeat regions comprise of ~200 amino acid residues (TOG domains), and each of these repeats consists of up to five HEAT motifs. The HEAT motifs are thought to be protein-protein interaction domains

that allow these proteins to interact with other proteins and, by virtue of these interactions, afford defined localization during specific stages, thereby modulating MT dynamics in a spatially and temporally controlled manner. The C-terminal non-repeat regions are likely to be responsible for MT-binding and centrosome, spindle pole body (SPB) or kinetochore localization. The number of TOG domain varies from two to five depending on the model organism. Human, *Xenopus* and *Drosophila* have five TOG domains, worms have three and yeasts have one or two TOG domains. The TOG domain arrays in XMAP215 family of proteins bind to free tubulin dimers, and promote their stable incorporation onto MT plus-ends while the basic linker regions separating the TOG domains promote association of the protein to the MT lattice (Ayaz et al., 2014; Ayaz et al., 2012; Currie et al., 2011; Fox et al., 2014; Widlund et al., 2011). Thus, TOG array usually promotes MT polymerization, and is required to generate mitotic spindles with appropriate spindle length in the different model systems studied, including *Drosophila* and *Xenopus* (Brittle and Ohkura, 2005; Currie et al., 2011; Reber et al., 2013).

At the centrosomes, XMAP215 orthologues function in MT stabilization and contribute to the formation of proper centrosomal asters (Cassimeris and Morabito, 2004; Cullen et al., 1999; Gergely et al., 2003; Popov et al., 2002). The XMAP215 family proteins generally function as MT polymerases at MT plus-ends to accelerate MT assembly by promoting growth (also called rescue) and suppressing catastrophe (Al-Bassam et al., 2012; Al-Bassam et al., 2006; Brouhard et al., 2008; Podolski et al., 2014; Widlund et al., 2011). *In vitro* TIRF microscopy has shown that the budding yeast Stu2 binds preferentially to MT plus-ends, and that the fission yeast Dis1 weakly associates with the MT lattice accumulating specifically at the growing MT plus-ends (**Fig. 1E2, Fig 2B & 2C**), while human chTOG has been found to accumulate at both the growing and shrinking MT plus-ends (**Fig. 1D2, Fig 2A**) (Matsuo et al., 2016; Podolski et al., 2014; Roostalu

et al., 2015; van Breugel et al., 2003). Budding yeast Stu2 and *Xenopus* XMAP215 have also been reported as MT-destabilizing factors at the plus-ends possibly by inducing catastrophe (Shirasu-Hiza et al., 2003; van Breugel et al., 2003). In humans, chTOG has not yet been reported to play a role in MT destabilization, but rather in spindle pole organization and spindle MT assembly (Cassimeris et al., 2009; Gergely et al., 2003). An *in vitro* study using TIRF microscopy has also shown that human chTOG functions as a MT polymerase and acts synergistically with another MT binding protein, TPX2, for nucleating MTs (Roostalu et al., 2015).

At kinetochores, the XMAP215 orthologs are required for regulation of kMT attachments (Garcia et al., 2002; Tanaka et al., 2005). Stu2 is required to form MTs from the kinetochores thereby facilitating the attachment of kinetochores to spindle MTs in budding yeast (**Fig. 1E1 & E2, Fig. 2B**) (Kitamura et al., 2010). A recent study has shown that Stu2 interacts directly with the kinetochore Ndc80 complex to control the stability of Ndc80-mediated kMT attachments at the dynamic MT tips (**Fig 1E2**); but the mode of their interaction is still unclear (Miller et al., 2016). It was observed that Stu2 function can either stabilize or destabilize Ndc80 mediated kMT attachments depending on the assembly or disassembly status of MT tips. These activities of Stu2 are defined by the level of tension imparted on the kinetochores (Miller et al., 2016). These studies strongly support the phenotypes observed (chromosome alignment defects and unattached kinetochores) in cells lacking Stu2 or human chTOG (Gandhi et al., 2011; Gillett et al., 2004; Kitamura et al., 2010; Kosco et al., 2001; Marco et al., 2013; Severin et al., 2001).

In fission yeast, the XMAP215 homologue Dis1 has been reported to associate with the loop domain of Ndc80 where it contributes to kMT attachments (**Fig. 1G1, Fig. 2C**) (Hsu and Toda, 2011; Tang et al., 2013). Also, in fission yeast, another XMAP215 homolog, Alp14, forms a stable complex with Alp7/TACC (Transforming Acidic Coiled-Coil family of proteins), another

MAP that binds to mitotic spindle MTs (**Fig. 1H1, Fig. 2C**) (Sato and Toda, 2007; Sato et al., 2004). Both Alp14 and Alp7 are recruited to spindle pole bodies and spindle MTs in an interdependent manner (**Fig. 1H2**). They are also recruited to mitotic kinetochores through the loop domain of Ndc80, where they have been reported to promote the formation of kMT attachments (**Fig. 1H2**) (Garcia et al., 2001; Tang et al., 2013; Tang and Toda, 2015). In humans, chTOG and the Alp7 homolog TACC3 interact with each other to form a chTOG-TACC3 complex, which is recruited to centrosomes and proximal mitotic spindles mediated by Aurora A kinase-dependent phosphorylation of TACC3 (**Fig. 1D2, Fig. 2A**) (Thakur et al., 2014). The chTOG-TACC3 complex associates with Clathrin, which is localized to kinetochore fibers and the mitotic spindle during mitosis, for its recruitment to the spindle MTs, and acts as an inter-MT bridge to stabilize kinetochore fibers (**Fig. 1D2, Fig. 2A**) (Booth et al., 2011; Compton, 2000; Royle, 2012). The components of chTOG-TACC3-Clathrin complex are dependent on each other to be recruited to spindle MTs (Booth et al., 2011). The mechanism of how Clathrin, together with chTOG and TACC3, regulates the stability of kinetochore fibers is still unknown, and further studies are necessary to address if this function is related with the kinetochore proteins including the Ndc80 complex.

XMAP215 orthologue in *Xenopus* and yeast, XMAP215/Dis1/Stu2 interacts with MT plus-end tracking protein EB1 (Kronja et al., 2009; Matsuo et al., 2016; Wolyniak et al., 2006) to regulate MT dynamics at the kMT interface (**Fig. 1E1 & 1E2, Fig. 1G1 & 1G2, Fig. 2B & 2C**). An *in vitro* study using purified Ndc80 complex from fission yeast has shown that the complex interacts with EB1 to track the growing MT plus-ends, but no detectable binding was observed between the complex and Dis1 (Matsuo et al., 2017). The studies described so far suggest the possibility that the Ndc80 complex could bind either directly to Dis1 or indirectly to Alp14 via the

mediation of Alp7 to contribute to the establishment of a dynamic kMT interface. It is noteworthy though that a direct interaction between Ndc80 and chTOG has not yet been observed in humans.

Analysis of MTs isolated from *Xenopus* egg extracts demonstrated an association between XMAP215 and Cyclin B1. Co-immunoprecipitation assay using MT fraction from *Xenopus* egg extract and HeLa cells determined that XMAP215 and its human orthologue chTOG interact with cyclin B1. *In vitro* co-sedimentation assay shows that cyclin B1 binds to MTs, and that its MT-binding is enhanced by XMAP215/chTOG (**Fig. 1D2**) (Charrasse et al., 2000). Cyclin B1 is recruited to unattached kinetochores via Ndc80 where it has been reported to be required for proper kMT attachments (**Fig. 1D1, Fig 2A**); but it is not clear how Cyclin B1 contributes to this function (Bentley et al., 2007; Chen et al., 2008). These observations point to a possibility where chTOG might play a role in facilitating Ndc80-mediated kMT attachment indirectly through cyclin B1.

In humans, chTOG is predicted to play a role in kMT attachments as observed for the yeast XMAP215 orthologs (**Fig. 1D1 & 1D2, Fig. 2A**) (Gergely et al., 2003; Meraldi et al., 2004; Miller et al., 2016). On the contrary, another study has suggested that this might not be the case as chTOG-depleted cells have stable kMT attachments under conditions of cold or high calcium (Cassimeris et al., 2009) Thus, further studies aimed at addressing the outstanding questions such as whether human chTOG interacts with Ndc80 either directly or indirectly (i.e. through other factors), and how the activities of chTOG and Ndc80 are coordinated, are required to shed light into the function of chTOG in kMT attachments in vertebrates.

(e) Astrin and SKAP

Astrin is a spindle-associated MAP (Chang et al., 2001; Mack and Compton, 2001) that localizes to the spindle MTs, spindle poles and outer kinetochores. Astrin is instrumental in normal mitotic

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progression, maintenance of chromatid cohesion and centrosome integrity (Dunsch et al., 2011; Gruber et al., 2002; Thein et al., 2007). Astrin forms a complex with + TIP, CLASP1, to promote kMT stability and chromosome alignment at the metaphase (Manning et al., 2010). Small kinetochore associated protein (SKAP), identified by proteome analysis of vertebrate kinetochores, has also been shown to localize to spindle MTs and spindle poles through its N-terminal region, and outer kinetochore through its C-terminal region (**Fig. 1C1, Fig. 2A**) (Dunsch et al., 2011; Schmidt et al., 2010; Wang et al., 2012). Experiments conducted by two independent groups, employing immunoprecipitation followed by mass spectrometry demonstrated that Astrin interacts with SKAP to form Astrin-SKAP complex (Dunsch et al., 2011; Friese et al., 2016; Kern et al., 2017; Schmidt et al., 2010). The targeting of the Astrin-SKAP complex to the bi-oriented kinetochores depends on the KMN network, Aurora B kinase activity and MT motor CENP-E (Huang et al., 2012; Manning et al., 2010; Schmidt et al., 2010; Wang et al., 2012). Co-sedimentation assay has shown that Astrin and SKAP bind directly to MTs individually as well as a complex (**Fig. 1C2**) (Schmidt et al., 2010). While the N-terminal region of Astrin binds to MTs through its interaction with SKAP, the C-terminal region is important for its kinetochore recruitment, the mechanism of which is unclear (Dunsch et al., 2011; Kern et al., 2017). In cells, the Astrin N-terminus (aa 1–693) was shown to localize to MTs and weakly to kinetochores. In contrast, the Astrin C-terminus (aa 694–1193), which lacks the SKAP binding site, localized to kinetochores, but not MTs (Kern et al., 2017); indicating that like Ska1, the MT-binding and kinetochore binding domains of Astrin-SKAP complex are independent and are separable (Kern et al., 2017). It has been reported that the MT-binding domain in SKAP stimulates the growth rate of MTs, possibly through a direct interaction with tubulin, suggesting that Astrin-SKAP complex has MT polymerase activity (Friese et al., 2016). This complex localizes to MT plus-ends where

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it interacts with both EB1 and EB3 through SxIP motif (Akhmanova and Steinmetz, 2010; Honnappa et al., 2009) to form a stable MT plus-end tracking complex (Tamura et al., 2015). *In vitro* TIRF microscopy shows that SKAP binds both to growing MT plus-ends in an EB1-dependent manner and also to the MT lattice (Friese et al., 2016; Wang et al., 2012). As previously mentioned, Astrin-SKAP may also stabilize MT plus-end dynamics at kinetochores through its interaction with CLASP1 (Manning et al., 2010). These studies suggest that this complex is required to form robust kinetochore fibers, and promotes stable kMT attachments (Dunsch et al., 2011; Thein et al., 2007).

Previous studies have shown that Astrin-SKAP complex interacts with dynein light chain DYNLL1, a component of the MT motor protein complex, cytoplasmic dynein. Thus, this complex possibly promotes dynein-mediated assembly and stability of the MT array, and the movement of kinetochores from MT plus ends toward the spindle poles (Dunsch et al., 2011; Sharp et al., 2000; Wittmann et al., 2001). This complex has also been shown to interact with a non-motor protein, NuMA (Nuclear Mitotic Apparatus protein) (Chu et al., 2016), another dynein binding partner, possibly to promote NuMA-mediated bundling of spindle MTs at the centrosomes, and contribute to spindle assembly (Haren et al., 2009; Merdes et al., 1996; Saredi et al., 1996). However, it is still unresolved if the interaction between Astin-SKAP and dynein contributes to the formation of robust kMT attachments.

How Astrin-SKAP complex coordinate in Ndc80-mediated kMT attachments was unclear until a recent *in vitro* study using the biochemically reconstituted Astrin-SKAP complex was carried out (Kern et al., 2017). In this study kinetochore cross-linking immunoprecipitation and mass spectrometry was used to show that Astrin-SKAP complex interacts with the Ndc80 complex in humans (**Fig. 1C1, Fig. 2A**). However, the details of this interaction are still not clear. Although

Astrin-SKAP complex docks at an unknown kinetochore location through Astrin's C-terminal region, the complex binds to Ndc80 through the N-terminal region of Astrin and to MTs through the SKAP MT-binding domain. However, unlike EB1, the MT-binding domain of SKAP lacks the SxIP motif that was found to promote the plus-end tracking and MT polymerization activity of EB1 (Friese et al., 2016). Further, the Astrin-SKAP complex binds to MTs synergistically with the Ndc80 complex to form an integrated and dynamic kMT surface that stabilizes kMT attachments. Astrin-SKAP complex displayed enhanced binding to MTs in the presence of a truncated version of the Ndc80 complex, (Ndc80 Bonsai) as is evident by the significant reduction in the apparent K_d of the Astrin-SKAP complex for MTs in the presence of Ndc80 Bonsai (1.4 µM) as compared to Astrin-SKAP alone (~3.2 µM) (Kern et al., 2017). Taken together, the Astrin-SKAP complex may facilitate processive interactions between the outer kinetochore and MT plus-ends as observed for other kinetochore-localized MT binding proteins including the Ska complex (or the Dam1 complex in yeast), and chTOG (Gergely et al., 2003; Lampert et al., 2010; Meraldi et al., 2004; Miller et al., 2016; Schmidt et al., 2012; Welburn et al., 2009). It has also been reported previously that SKAP physically interacts with Mis13/Dsn1 subunit of the kinetochore Mis12 complex, a component of the KMN network to serve a link to dynamic spindle MTs (Wang et al., 2012).

5. Conclusions

All the afore-mentioned kinetochore proteins that function as MAPs including Cdt1, the Ska complex, chTOG and the Astrin-SKAP complex coordinate with the outer kinetochore complex Ndc80 in re-shaping the major MT binding interface of human metaphase kinetochores. In this manuscript, we present a detailed overview of the functional relationship of these kinetochore MAPs with the Ndc80 complex and their targeting to the kMT interface in diverse model systems, including yeast and humans (**Figures 1 & 2**, see details in legends). Briefly, in yeast, the

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stabilization of kMT attachments by the Ndc80 complex is promoted by XMAP125 homologues (Stu2 in budding yeast, Dis1 and Alp14 in fission yeast, **Fig. 1E1 & 1E2, Fig. 1G1 & 1G2, Fig. 2B & 2C**) and the Dam/DASH complex (in budding yeast, **Fig. 1F1 & 1F2, Fig. 2B**). The Dam/DASH complex plays a role in kMT attachments by interacting with the loop domain and unstructured tail of Ndc80 (**Fig. 1F1, Fig. 2B**). Alp7/Alp14 and Dis1 in fission yeast also promote kMT stabilization through interaction with the loop domain of Ndc80 (**Fig. 1H1, Fig. 2C**). In mammals, the Ndc80-mediated kMT attachment is enhanced by Cdt1 and the Ska complex in conjunction with the loop domain of Ndc80 (**Fig. 1A1 & 1A2, Fig. 1B1 & 1B2, Fig. 2A**). Ska also helps in kMT stabilization by interacting with the N-terminal unstructured tail of Ndc80 (**Fig. 1B1, Fig. 2A**). In contrast, it is still unclear how human chTOG and the Atrin-SKAP complex associate with the Ndc80 complex to enhance the stability of kMT attachments. Moreover, how these different MAPs coordinate with each other and with the Ndc80 complex, the master regulator, is an arena for further research that will most likely unveil several layers of mechanistic aspects involved in the formation, maturation and stabilization of kMT attachments during mitosis. Further, the details of whether these different kinetochore MAPs team up to stabilize kMT attachments or act independently of each other is still elusive. In addition, other MT-binding kinetochore components such as the kinetochore motors CENP-E and dynein, and the MT plus-end tracking proteins including EB1, CLIP-170, and CLASP1/2 also contribute to the formation of stable kMT attachments (Akhmanova et al., 2001; Brunner and Nurse, 2000; Cheeseman and Desai, 2008; Nakamura et al., 2001; Santaguida and Musacchio, 2009; Schuyler and Pellman, 2001). Studies unraveling whether these kinetochore proteins contribute to kMT attachment in coordination with the Ndc80 complex or the kinetochore MAPs will facilitate a detailed and

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comprehensive understanding of how kinetochores are stably coupled to the ends of dynamic MTs to prevent chromosome mis-segregation.

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The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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Figure legends

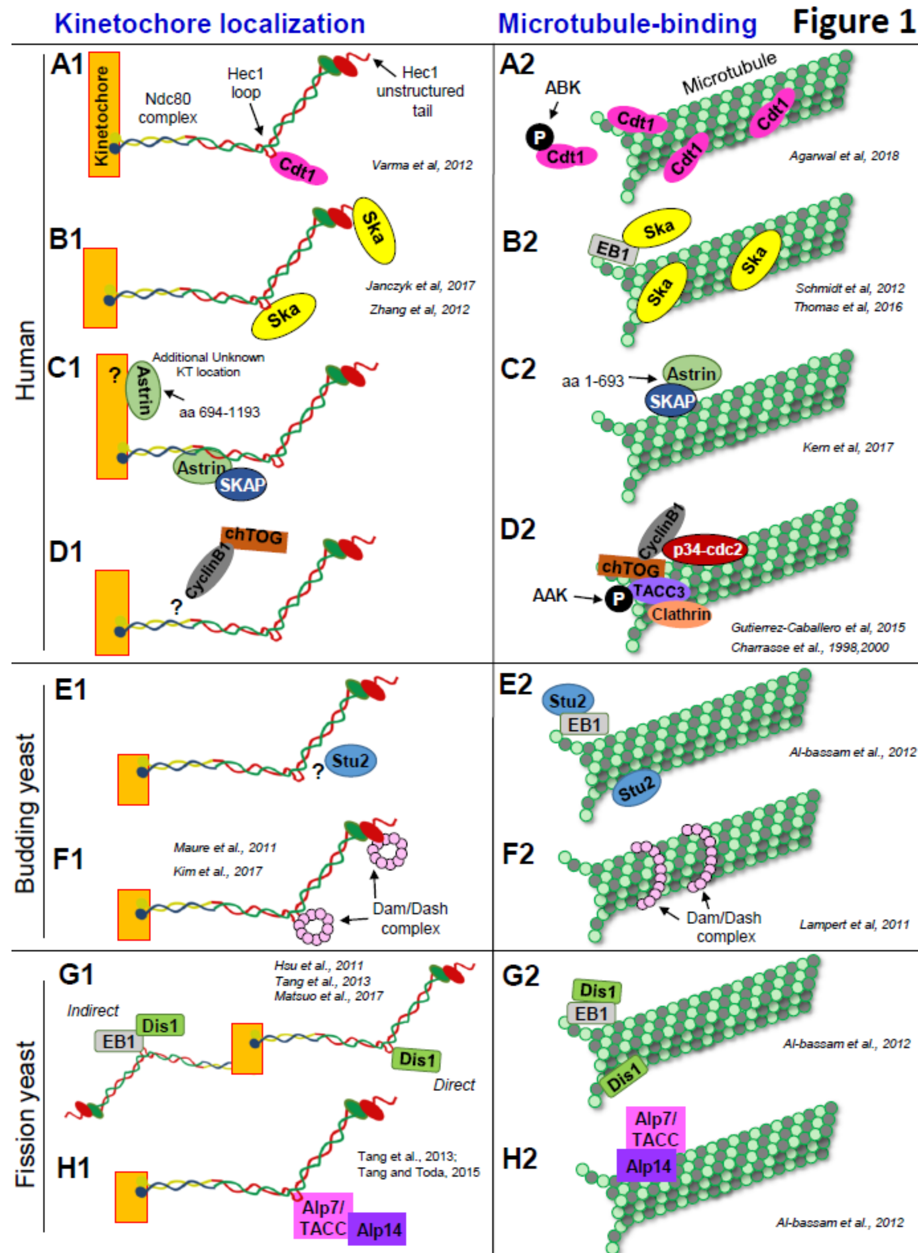

Figure 1: A detailed view of various kinetochore MAPs across phyla, dissociating their kinetochore and microtubule localization and functions. A schematic representation of several microtubule associated proteins (MAPs) ranging from yeast to humans based on their function or classification within a family. Multiple interactions are shown to depict the localization of each

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MAP either directly or indirectly at both kinetochores and MTs. Kinetochore localization specifically refers to the ability of indicated MAPs to bind to the Ndc80 complex of the KMN network unless otherwise specified. Human Cdt1 localizes to kinetochores with the help of the loop domain of the Ndc80 complex (**A1**) while it binds to MTs directly (**A2)**. MT-binding of Cdt1 is regulated by Aurora B kinase phosphorylation (**A2**). The binding to human Ska complex to kinetochores is thought to require the loop domain of Ndc80 (**B1**). Ska binds MTs directly while it has been reported to bind to the plus-ends with the help of the + TIP, EB1 (**B2**). In the context of XMAP215 family members, fission yeast Dis1 have been shown to be directly dependent on the loop domain to localize to kinetochores (**E1**) while Alp14 localization to the loop is dependent on its binding partner, Alp7/TACC (**H1**). However, for budding yeast Stu2, the region of Ndc80 that is required for kinetochore localization is not clear (**E1**). As far as MT-binding is concerned, it is well established that all XMAP215 members, including chTOG in humans bind directly to MTs (**E2**, **G2** & **H2**); For Stu2, binding to plus-ends is also dependent on the yeast EB1 homologue (**E2**) . For chTOG, MT-binding has been shown to be dependent on another MAP, TACC3, and is also thought to require Cyclin B/Cdc2 function (**D2**). However, it is not clear yet if Cyclin B binds directly to the Ndc80 complex to recruit chTOG to kinetochores (**D1**). Human Astrin binds to MTs through its binding partner, SKAP (**C2**). It is not clear at this point whether Astrin localizes to kinetochores by binding directly to the Ndc80 complex or to another receptor (**C1**). Finally, the Dam1 ring complex in budding yeast binds MT directly (**F2**) and localizes to the kinetochore by binding to multiple regions of Ndc80, including the loop domain (**F1**). The proteins and protein complexes, which are color-coded with those in Fig. 2, are drawn in arbitrary shapes for the purpose of depiction only and are not to the scale. The Ska complex constitutes three subunits, Ska1, 2 and 3, but for brevity, clarity and ease, the complex is represented as a singular unit (yellow

ovals). Additionally, the region within Ndc80, where these MAPs bind are not precisely marked because the exact details of the interface of interaction in many cases has not been resolved.

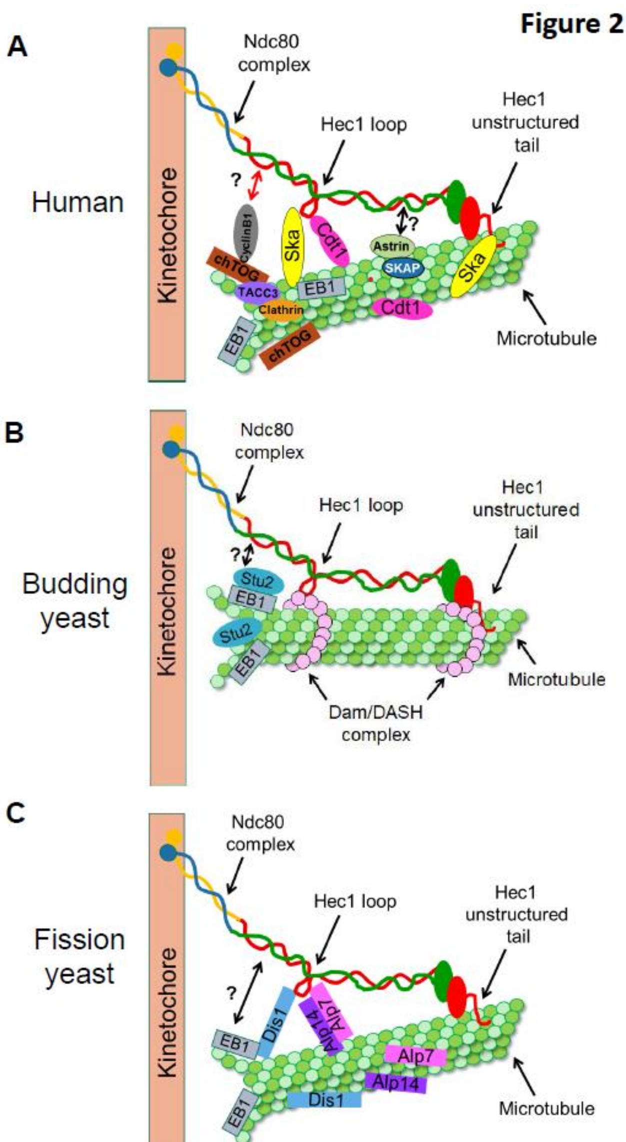

Figure 2: A summary of MAP function at the kMT interface that is required for the stabilization of kMT attachment in yeast and humans. Formation of robust kMT attachments is enabled by MAPs recruited to kinetochores through the interaction with the Ndc80 complex. **A.**

In humans, Cdt1 binds to Ndc80 through its loop domain. Ska also directly binds to Ndc80 through both the loop and CH domains. Both Cdt1 and Ska enhance the formation of Ndc80-mediated stable kMT attachments at the kMT interface. The C-terminal of Astrin in complex with SKAP binds to a yet unidentified region of kinetochore Ndc80, while the Astin-SKAP complex binds to MT through the SKAP MT-binding domain. XMAP125 homologue chTOG binds to MTs either directly or by the mediation of another MAP, TACC3. The interaction between chTOG and the Ndc80 complex is likely indirect, mediate through Cyclin B1. Cyclin B1 is recruited to kinetochores by Ndc80, but it is also not yet known if they exhibit a direct interaction with each other. chTOG, TACC3 and clathrin have been found to be important to recruit each other to spindle MTs. **B**. In budding yeast, the Dam1/DASH complex forming oligomers and/or ring structures around a MT after being recruited at kinetochores by binding to the loop and CH domains of Ndc80. XMAP125 homologue Stu2 directly binds to kinetochore Ndc80 and the MT-tracking protein EB1; but the details of how Stu2 interacts with Ndc80 is yet unclear. **C**. In fission yeast, XMAP125 homologue Dis1 is loaded on to kinetochores through a direct interaction with the loop domain where it interacts with EB1 located at the MT plus-end. EB1 interacts with Ndc80 *in vitro,* but the functional relevance of this interaction is still unclear *in vivo*. The other XMAP125 homologue Alp14 forms a complex with Alp7/TACC and is recruited to the kinetochore by an interaction with the Ndc80 loop domain. Black arrows indicate the interaction between proteins but the site on Ndc80 is not yet established. Red arrows indicate that the interaction between two proteins is not yet known.

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