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Review

Dynein at the kinetochore: Timing, Interactions and Functions

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ABSTRACT

Kinetochores have been proposed to play multiple roles in mitotic chromosome alignment, including initial microtubule (MT) capture, monitoring MT attachments, prometaphase and anaphase chromosome movement and tension generation at metaphase. In addition, kinetochores are essential components of the spindle assembly checkpoint (SAC), and couple chromosome alignment with SAC silencing at metaphase. Although the molecular details of these activities remain under investigation, cytoplasmic dynein has been implicated in several aspects of MT and SAC regulation. Recent work clarifies the contribution of dynein to MT interactions and to events that drive anaphase onset. This review summarizes these studies and provides new models for dynein function.

Contents

1. Introduction

Kinetochores are transient, chromosome-associated protein assemblies that form during mitosis to mediate force-producing interactions with MTs, MT binding proteins and additional components required for mitotic regulation [1,2]. Perhaps the most distinctive feature of kinetochores in higher eukaryotes is the trilaminar plate structure revealed by EM analysis [3]. Functional roles in providing an interface between chromatin and MTs are suggested by the structures adjacent to the inner and outer plates. Roles in sensing tension and stretch are suggested by the increasing distance between inner and outer plates at metaphase. Furthermore, kinetochores are implicated in depositing signaling molecules at the spindle midzone after anaphase onset, dictating the position of

Abbreviations: IFM, immunofluorescence microscopy; MT, microtubule; SAC, spindle assembly checkpoint.

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the cytokinetic cleavage furrow [4]. How kinetochores orchestrate these distinct activities has only recently been deciphered.

Among the most challenging aspects of kinetochore analysis is the growing number of proteins that accumulate there and the hierarchy of these proteins in the kinetochore structure [5]. Approximately 80 proteins have been identified as core kinetochore components and the organization of many of these proteins is conserved among eukaryotes. Some features of the outer kinetochore are more complicated in higher eukaryotes including components of the cytoplasmic dynein pathway and the RZZ complex (*rod*, *zw10*, zwilch) [2]. The reasons for this additional complexity in higher eukaryotes are not known, but could include: (1) increased chromosome number, (2) the rapid dispersal of chromosomes into the cytoplasmic volume after nuclear envelope breakdown and (3) the need to initiate MT-chromosome interactions *de novo* in a larger space.

An intriguing component of the more highly-elaborated outer kinetochore is the MT motor cytoplasmic dynein. Dynein is a multisubunit, minus end-directed motor responsible for many aspects of intracellular motility, including organelle transport [6,7], retrograde axonal transport [7], and cell migration [8]. Dynein is also implicated in multiple mitotic functions. During the G2/M transition, dynein releases from interphase cargos and localizes to three novel mitotic loci: (1) the cell cortex, (2) spindle poles, and (3) kinetochores. There is consensus that polar and cortical localizations of dynein reflect roles in spindle formation/integrity and spindle rotation/positioning [9–13]. In contrast, the specific contributions of dynein at kinetochores remain controversial [14]. Kinetochore dynein has been implicated in MT attachment [15–17], chromosome movement [18–20] and regulation of the SAC [21–23]. In addition, several proteins have been proposed to interact, directly or indirectly, with kinetochore dynein as a means to regulate dynein targeting and/or function. The mechanisms that coordinate these interactions/functions are not well understood.

2. When is dynein at the kinetochore?

Immunofluorescence microscopy (IFM) studies with dynein antibodies demonstrate that dynein localizes initially to kinetochores during prometaphase [15–17,24]. Dynein accumulates at kinetochores prior to MT attachment (Fig. 1), and displays exaggerated recruitment after MT depolymerization [17,24]. The latter suggests that dynein is a component of the fibrous corona implicated in sensing MT attachment and providing feedback to the kinetochore. The timing of kinetochore localization together with dynein inhibition studies suggest that dynein is involved in initial interactions with MTs and in early aspects of chromosome movement during prometaphase [19,24–27]. Interestingly, as chromosomes achieve bipolar attachment and approach the metaphase plate (Fig. 1), kinetochore dynein becomes less prominent by IFM analysis [28]. This reduction in kinetochore dynein coincides with enhanced dynein labeling along spindle fibers and spindle poles [22,23]. This transition from kinetochores to spindle fibers is consistent with recent models that link dynein to checkpoint silencing at metaphase [2,21–23,29,30]. After alignment, dynein is undetectable at kinetochores [15,16,23], and is not detected on kinetochores throughout anaphase and cytokinesis (Fig. 1). Although the loss of dynein labeling at metaphase kinetochores could reflect antibody inaccessibility issues, dynein is detected on spindle fibers and at spindle poles after alignment [23]. Another possibility is that the enhanced motility of dynein after alignment reduces dynein accumulation to very low levels. Consistent with this possibility, live imaging of dynein in *Drosophila* reveals kinetochore labeling that persists into anaphase [18]. Additional live imaging studies will be needed to determine the degree of dynein binding at kinetochores after anaphase onset.

3. Proposed kinetochore binding partners

One approach that has been used to address the function of kinetochore dynein involves manipulating candidate binding partners. A growing number of proteins have been proposed to recruit or mediate binding of dynein to the kinetochore, including: dynactin, *zw10*, LIS-1, nudE/EL, nudC and Spindly. This section summarizes the evidence linking each to kinetochore dynein (Table 1).

3.1. Dynactin

Dynactin is a multi-subunit complex first identified as a dynein cofactor capable of stimulating dynein-based transport events *in vitro* [31–34]. A direct interaction between the dynein intermediate chains (ICs) and the p150^{Glued} subunit of dynactin implicates dynactin as a dynein cargo receptor [35,36]. Consistent with this possibility, dynactin is involved in most examples of dynein-driven

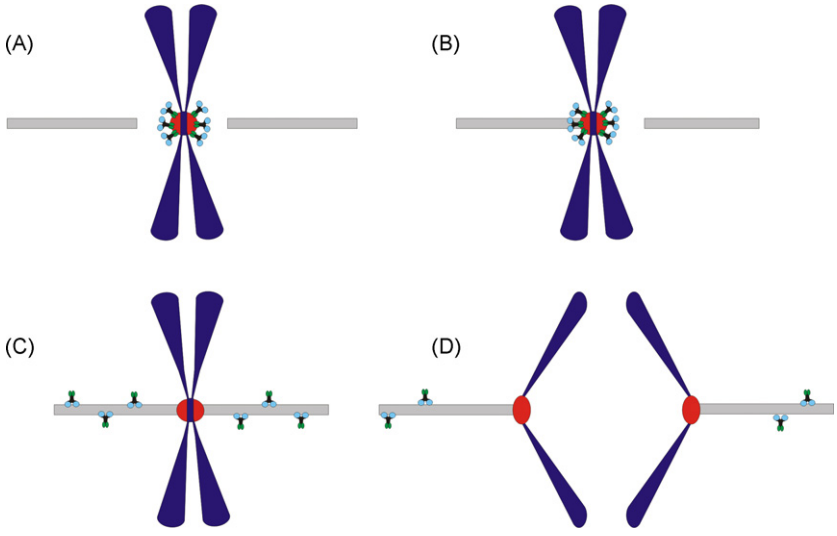

Fig. 1. Changes in kinetochore dynein during mitosis. Dynein is initially recruited to kinetochores before MT attachment (A) and remains at kinetochores during the process of mono-orientation and association with the spindle (B). After bioriented MT attachments are achieved (C), dynein undergoes poleward movement and labels K-fibers and spindle poles. By anaphase onset (D), dynein is prominent on spindle fibers but undetectable at kinetochores.