Model of Chromosome Motility in *Drosophila* Embryos: Adaptation of a General Mechanism for Rapid Mitosis

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ABSTRACT During mitosis, ensembles of dynamic MTs and motors exert forces that coordinate chromosome segregation. Typically, chromosomes align at the metaphase spindle equator where they oscillate along the pole-pole axis before disjoining and moving poleward during anaphase A, but spindles in different cell types display differences in MT dynamicity, in the amplitude of chromosome oscillations and in rates of chromatid-to-pole motion. *Drosophila* embryonic mitotic spindles, for example, display remarkably dynamic MTs, barely detectable metaphase chromosome oscillations, and a rapid rate of "flux-pacman-dependent" anaphase chromatid-to-pole motility. Here we develop a force-balance model that describes *Drosophila* embryo chromosome motility in terms of a balance of forces acting on kinetochores and kMTs that is generated by multiple polymer ratchets and mitotic motors coupled to tension-dependent kMT dynamics. The model shows that i), multiple MTs displaying high dynamic instability can drive steady and rapid chromosome motion; ii), chromosome motility during metaphase and anaphase A can be described by a single mechanism; iii), high kinetochore dynein activity is deployed to dampen metaphase oscillations, to augment the basic flux-pacman mechanism, and to drive rapid anaphase A; iv), modulation of the MT rescue frequency by the kinetochore-associated kinesin-13 depolymerase promotes metaphase chromosome oscillations; and v), this basic mechanism can be adapted to a broad range of spindles.

INTRODUCTION

Chromosome segregation depends upon the action of the mitotic spindle, a protein machine that uses ensembles of mitotic motors and MT dynamics to capture chromosomes consisting of duplicated sister chromatids and align them at the metaphase spindle equator and then to move sister chromatids to opposite spindle poles during anaphase (1–3). The sister chromatids are attached to the spindle by kts, protein complexes assembled on centromeric DNA that consist of several distinct layers as observed by EM (4,5), and which bind to the plus ends of a subset of spindle MTs called kMTs whose minus ends are also linked to the poles (6).

KMTs play important roles in chromatid motility, and in many systems they are very dynamic. For example, during metaphase, kMTs display dynamic instability (7) at their plus ends and they also exhibit motor-dependent poleward flux, in which the MT polymer lattice persistently translocates poleward as tubulin subunits undergo net addition onto the dynamic MT plus ends and net dissociation from their pole-associated minus ends (8,9). This dynamic behavior contributes to the oscillations of congressed metaphase chromosomes along the pole-pole axis, a process called ''directional instability'' (10). During anaphase A, kMTs continue to undergo poleward flux as tubulin subunits dissociate at their pole-associated minus ends, and, if subunit addition at the kt ceases or slows down, the kMTs can then shorten and drag the disjoined chromatids poleward (11–13). In many systems, this ''flux mechanism'' for anaphase A is supplemented or replaced by a ''pacman'' mechanism, in which the kinetochores actively ''chew'' their way to the poles by depolymerizing kMTs at their plus ends, dragging the attached chromatids poleward (14–18). While kMTs exert the forces that underlie both metaphase chromosome oscillations and anaphase A chromatid-to-pole motility, a second subset of MTs, the ipMTs, drive spindle elongation during anaphase B. Modifications of these basic events occur in many cell-types and there exists significant variability in the rates of chromosome motility, in the magnitude of the oscillations associated with directional instability, in the relative contributions of the flux and pacman components of anaphase A, and in the relative contributions of anaphase A and B to chromosome segregation, within different systems (11–15,18,19).

The *Drosophila* syncytial blastoderm stage embryo (cycles 10–13) is a veritable mitotic factory packed with mitotic spindles whose hallmark is rapid mitosis (14,15,18,20,21). The syncytium contains the order of a thousand spindles lying just under the cortex that are derived from the single nucleus of the fertilized egg through a stereotypical series of mitoses and nuclear migrations. Each spindle assembles as the nuclear envelope fenestrates during prometaphase when eight pairs of sister chromatids are captured and maneuvered

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Abbreviations used: MT, microtubule; kt, kinetochore; kMT, kinetochore microtubule; ipMT, interpolar microtubule; EM, electron microscopy; FRAP, fluorescence recovery after photobleaching.

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onto the equator of the ~10 μm long metaphase spindle, where they are held in a relatively static state, displaying no obvious directional instability (Fig. 1 *A*) (3,13). Anaphase A chromatid-to-pole motility depends on a combined ''flux-pacman mechanism'' and is remarkably fast (0.1 μm s^{-1}) (14,18). Once chromatid-to-pole motion is essentially complete, anaphase B onset is triggered by the suppression of poleward flux within ipMTs, which allows persistently sliding ipMTs to exert forces that drive spindle pole separation at a similar fast rate (14,21). The spindle MTs are highly dynamic, displaying a turnover half-time of ~5 s in FRAP experiments, independent of the position or phase of photobleaching ((21) and D. Cheerambathur and J. M. Scholey, unpublished results) and fluxing poleward at 0.05 μm s^{-1} before anaphase B onset (14). This rapid turnover rate is plausibly due to dynamic instability of all subsets of spindle MTs, leading to the question ''how can MTs that display rapid turnover and switch frequently between fast growth and shrinkage, drive steady and rapid motility?'' Computational modeling using systems of force-balance and rate equations suggests that highly dynamic ipMTs can drive steady, linear pole-pole separation during anaphase B (21), and below we use similar modeling approaches to determine the feasibility of driving rapid, steady chromatid-to-pole movements using highly dynamic kMT tracks.

Several mitotic motors have been implicated in chromosome motility during metaphase-anaphase A in *Drosophila* embryo spindles. For example, dynein and members of the kinesin-7 (cenpE), kinesin-3 (KLP38B), and kinesin-13 (KLP59C) families (22) appear to act on kts or chromosome arms to contribute to chromosome positioning at the metaphase equator, whereas the rapid, flux-pacman-driven chromatid-to-pole motion during anaphase A is thought to be driven by a kinesin-13-dependent mechanism in which KLP10A depolymerizes kMTs at the spindle poles to drive poleward flux, whereas KLP59C depolymerizes kMTs at the kinetochore to drive ''pacman'' motility (18,20,23,24). In this mechanism, dynein located at the kinetochores is thought to assist KLP59C by inserting the plus ends of kMTs into the kinetochore structure to facilitate KLP59C-mediated depolymerization (5,18,20,25).

Although some aspects of chromatid motility that are used in *Drosophila* embryos are likely to be widely employed among different cell types, other features may represent adaptations for rapid motility. For example, evidence is accumulating from a number of systems in support of the hypothesis that a kinesin-13 depolymerase located at the spindle poles plays a significant role in driving poleward flux (26–28). In contrast, most studies on the role of kinesin-13 and dynein on kinetochores has focused on the role of these motors in error-correction mechanisms and in the spindle assembly checkpoint, rather than in chromatid motility per se (29–33). Thus, it is possible that the KLP59C and dynein-based ''pacman'' mechanism used in *Drosophila* embryos is a functional adaptation that facilitates rapid motility concordant with the rapid rates of mitosis observed, a possibility that can be explored using modeling.

Two pioneering quantitative models have recently been proposed to describe chromosome motility (34–36). In the first, a force-balance model of the kinetochore was successfully used to describe the forces that drive metaphase chromosome oscillations and directional instability, based on a ''Hill-sleeve'' structure in which the kinetochore contains ''sleeves'' that bind kMTs on their inner surface (34,37,38). However, in this study, the identity and mechanism of action of the relevant kinetochore motors were not examined. A different theoretical approach was used to describe the positioning of metaphase kinetochores in the budding yeast spindle (35,36), but in that study the mechanism by which kinetochores attach to spindle MTs and remain attached under varying force regimes was not addressed.

Here, we develop a mathematical force-balance model of chromosome motility that describes the dynamics of a pair of sister kinetochores and their associated kMTs during metaphase and anaphase A in *Drosophila* syncytial blastoderm embryos. The model is based on a kinetochore-MT interface as drawn in Rogers et al. (18), Maiato et al. (5), Maddox et al. (13), and Rieder and Salmon (25), and incorporates the concerted action of force generators coupled to MT-dynamics. The model includes the dynamics of kMT and its modulation by enzymes and forces; the forces generated by antagonistic and complementary enzymes and polymers at the kinetochores and poles; a simplified mechanistic description of the centromeric cohesin bonds between sister chromatids; polar ejection forces; and a force-balance between the forces acting on kts and viscous drag forces (34–36). By varying the model parameters, we provide a good description of metaphase-anaphase A kt behavior in *Drosophila* embryos and also in various other cell-types based on the action of mitotic motors and MT dynamics, without the need to invoke additional poorly characterized structures such as ''Hill sleeves''. The model demonstrates: 1), that multiple highly dynamic and transiently attached kMTs can drive steady, accurate chromosome movements; 2), that kts can maintain persistent attachment to a spindle pole despite the high dynamicity of the kMT plus ends and the presence of several force generators; 3), that the low amplitude and frequency of metaphase chromosome oscillations in *Drosophila* embryonic spindles may be due to high dynein activity at the kinetochores, 4), that the action of the kinesin-13 depolymerase KLP59C promotes metaphase oscillations; and finally 5), explores the generality of the proposed mode of action of the *Drosophila* pacman motor in other organisms.

MODEL

In this section, we first describe the model variables and equations in a simplified configuration as shown in Fig. 1, *B* and *C*, where only a single kMT is shown bound to the kinetochore. In the final subsection, we generalize the model to account for a realistic configuration of the