Chromosome Oscillations in Mitosis

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The motion and positioning of chromosomes during eukaryotic cell division is investigated theoretically. We perform a self-contained analysis where the motion of mono-oriented chromosomes results from the competition between the kinetochore and chromokinesin motors on the chromosome arms. We show that the interplay between the asterlike morphology of the mitotic spindle and the collective dynamics of motors accounts for chromosome motion, positioning, and congression. In particular, the characteristic oscillations of chromosomes observed *in vivo* arise naturally within this description.

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Successful cell division requires a tight regulation of chromosome motion via the activity of molecular motors. Many of the key players at the origin of the forces generating the movement have been identified, but their spatial and temporal organization remains elusive [1]. In the prometaphase of animal cells, mono-oriented chromosomes (associated with a single microtubule aster) periodically switch between phases of poleward and away from the pole movement. This oscillatory movement, which persists during chromosome congression, metaphase, and early anaphase [2–5], is a signature of the forces acting on the chromosome. Its quantitative description can thus help understanding the forces driving mitotic movements.

It is widely accepted that the motion of a mono-oriented chromosome arises from the tug of war between two main forces, namely, the poleward directed kinetochore force and the polar ejection force generated collectively by chromokinesin motors [4–7]. The poleward (P) force exerted by the kinetochore is thought to be due to cytoplasmic dyneins [8] and microtubule (MT) depolymerization in the kinetochore [9]. Chromokinesin motors associated with the chromosome arms [10] move toward the plus end of MTs [11] and generate the polar ejection force driving the chromosome away from the pole (AP) [5,12]. Several models have been proposed to explain the oscillatory behavior of chromosomes from the generation of forces within the kinetochore [4,6,7,13]. We adopt a different theoretical approach and analyze the nature of the polar ejection forces. Our description accounts for the crucial and experimentally proven role of chromokinesins, and explains why their inhibition suppresses chromosome oscillations [5].

The collective behavior of molecular motors can generically give rise to dynamical instabilities [14] which have been observed in biological [15] and biomimetic [16] systems. Nevertheless, the existence of a dynamical instability does not necessarily imply periodic oscillations in space if it is not coupled to spatial information. While the AP force has been shown to depend on the MT density in the spindle [17], the precise link between them remains unknown. We argue that the chromosome positional information is provided by the interaction of chromokinesins with the astral MTs in the spindle which, due to their asterlike distribution, constitute a position-dependent substrate for motor binding. In order to precisely assess the role of chromokinesins, we analyze the balance of forces on a mono-oriented chromosome [Fig. 1(a)].

Chromosome motion occurs at length and velocity scales for which inertial effects are negligible (low Reynolds number). The forces acting on the chromosome are: the poleward force F_K created by the kinetochore, the polar ejection force F_{AP} due to chromokinesin motors, and a friction force opposing motion [Fig. 1(b)]. Force balance reads

$$F_{\mathrm{AP}} - F_K - \xi \dot{r} = 0, \tag{1}$$

where $\dot{r} \equiv dr/dt$ is the chromosome velocity. Any mismatch between the kinetochore force and the polar ejection force induces chromosome motion, characterized by a friction coefficient ξ. Friction occurs both on chromosome arms and within the kinetochore, where it likely arises from MT dynamics and the activity of molecular motors [18]. As a first step to elucidate the consequences of chromokinesin collective behavior on chromosome motion, both the kinetochore force and the global friction parameter are taken as constant.

It has been shown that the kinetochore does not contribute to the AP force [19,20], which is created by the binding and displacement of chromokinesin motors on the MT aster [5] [Fig. 1(a)]. We consider N chromokinesins permanently attached to the chromosome arms, which stochastically attach to and detach from MTs with average binding and unbinding rates k_b and k_u, respectively [Fig. 1(c)]. At one given time, only an amount n of all available chromokinesins is bound to the MTs in the aster and is able to participate to the AP force. Generically, the time evolution of the number of bound chromokinesins reads

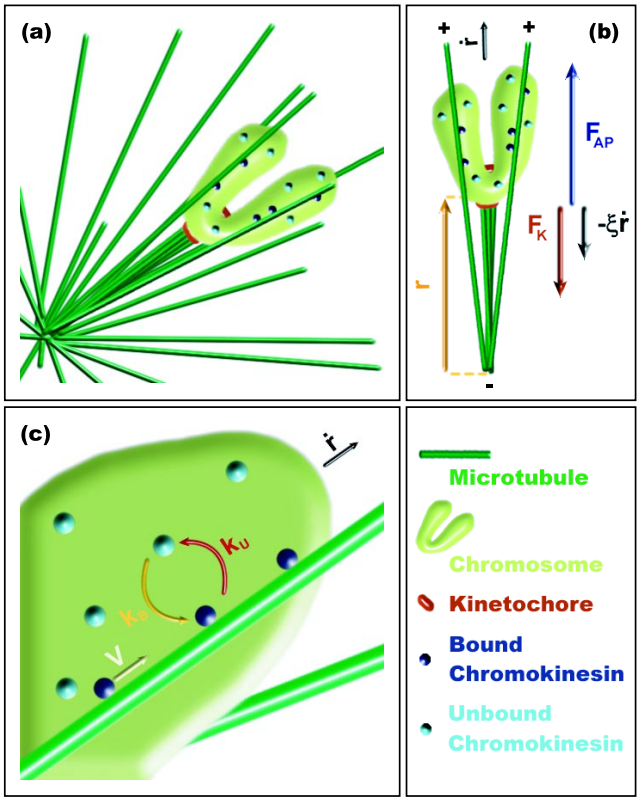

FIG. 1 (color online). (a) Sketch of a MT aster (dark green) interacting with a single chromosome (light green). The kinetochore (red) is connected to the pole through a bundle of MTs. Chromokinesin motors on the chromosome arms may be bound to a MT (dark blue dots) or unbound (light blue dots). (b) Forces driving chromosome motion: the kinetochore poleward force F_K (red), the polar ejection force created by the bound chromokinesins F_{AP} (dark blue), and the friction force opposing motion $-\xi\dot{r}$ (black). The chromosome position relative to the pole (orange arrow) is r and its velocity is $\dot{r}$. The $(+)$ and $(-)$ ends of MTs are indicated. (c) Binding or unbinding kinetics of chromokinesin motors, with rates k_b and k_u, respectively. In the bound state, chromokinesins move toward the plus end of MTs with a velocity V.

$$\frac{dn}{dt} = k_b(N-n) - k_u n. \tag{2}$$

The binding rate k_b takes into account the probability of encounter between a motor and a MT. In a monopolar spindle the MT density, $\rho_{MT}(r)$, decreases away from the pole, so that the binding rate $k_b(r) = k_b^{(0)} S_{ch}\rho_{MT}(r)$ is a decreasing function of the chromosome position r ($k_b^{(0)}$ being the attachment rate of chromokinesins onto a neighboring MT and S_{ch} the effective chromosome surface that interacts with spindle MTs). For an ideal isotropic monopolar spindle composed of M MTs, the MT density decreases as $\rho_{MT}(r) = M/4\pi r^2$. Note that neither the P nor the AP forces depend explicitly on the chromosome position. All spatial information is contained in the binding rate $k_b(r)$ and reflects the morphology of the MT spindle.

The velocity V and unbinding rate of a bound motor are strongly influenced by the motor load. If the n bound chromokinesins are independent from one another, they equally contribute to the total ejection force F_{AP} on the chromosome, so that each motor applies (and experiences according to Newton's third law) a force F_{AP}/n. Motor unbinding is a stochastic process which rate increases exponentially with an applied load [21,22]: $k_u = k_u^{(0)} \exp[F_{AP} a/n k_B T]$, where $k_u^{(0)}$ is the unbinding rate at vanishing load, a is a phenomenological length, and $k_B T$ is the thermal energy. The velocity V of a bound motor decreases with a force opposing motion. For simplicity, we adopt the linear relationship $V = V_0(1 - F_{AP}/n f_s)$ [23], where f_s is the motor stall force.

Identifying the chromosome velocity $\dot{r}$ with the chromokinesins velocity V on MTs and combining the equations above, we obtain a dynamical system for the evolution of $\{n, r\}$, which reads

$$\begin{aligned} \dot{n} &= k_b(r)(N-n) - k_u^{(0)} \exp\left(f\frac{n_s + n_\xi}{n + n_\xi}\right) n, \\ \dot{r} &= V_0 \frac{n - n_s}{n + n_\xi}. \end{aligned} \tag{3}$$

Three dimensionless parameters control the dynamical state of the chromosome: $n_s/N \equiv F_K/N f_s$ is the relative amount of bound motors at which AP and P forces exactly balance, $n_\xi/N \equiv \xi V_0/N f_s$ characterizes the effect of chromosome friction, and $f \equiv f_s a/k_B T$ quantifies the sensitivity of motor unbinding to an external load.

Close to the pole, the MT density is high; many chromokinesins attach to spindle MTs and produce a large force that moves the chromosome away from the pole. Far from the pole, MTs are scarce; chromokinesins detach and the chromosome moves poleward due to the kinetochore force. Somewhere in between, there exists a fixed point where the system remains still. It corresponds to a number of bound chromokinesins n_s and to a chromosome position r_s, given implicitly by $k_b(r_s) = k_u^0 e^f n_s/(N - n_s)$, where chromokinesin attachment and detachment fluxes exactly compensate.

In case the fixed point is stable, the chromosome stalls at a distance r_s from the pole. If it is unstable, the analysis of the full nonlinear dynamical system [Eq. (3)] shows that the chromosome undergoes sustained periodic oscillations [24]. Stability is lost when the fluxes of motor attachment and detachment are not able to compensate, so that the detachment of one or a few motors sufficiently increases the force per remaining motor to induce the dramatic unbinding of them all. Linear perturbation analysis around $\{r_s, n_s\}$ shows that the fixed point is unstable for a range of parameters satisfying

$$\frac{n_\xi}{N} < \frac{n_s}{N}\left(f - 1 - f\frac{n_s}{N}\right). \quad (4)$$

The value of f is not known for chromokinesin but it is estimated to be $f \simeq 2$ for conventional kinesin [16,25]. The range of stability for $f = 2$ is shown in Fig. 2.

It has been experimentally observed that vastly reducing the number of chromokinesins ($\gtrsim 90\%$ reduction) leads to the disappearance of the oscillations of mono-oriented chromosomes and their collapse onto the centrosome, while not preventing bi-oriented chromosomes to congress to the metaphase plate [5]. Within our framework, a reduction of the total number N of available chromokinesins has the effect of moving the system along a straight line in the parameter space (arrow in Fig. 2), and to eventually exit the oscillatory regime at $N_c \equiv f n_s^2/[(f-1)n_s - n_\xi]$. Forces in mitosis are typically of order 500 pN [26]. With this estimate for the kinetochore force and $f_s \simeq 3$ pN [12], along with typical values for the average chromosome-to-pole distance and the amplitude and period of the oscillations in Newt lung cells [3,4], we estimate a total number $N \simeq 1500$–5000 of chromokinesins on the chromosome arms, and a critical number $N_c \simeq 600$–700 above which oscillations are expected. The estimated value for N is consistent with the measured chromokinesin density on chromosome arms [12]. We thus predict that inhibition of 60% to 90% of the chromokinesins would be sufficient to suppress oscillations. This shows that a significant number of chromokinesins may remain attached to the arms of nonoscillating chromosomes. When such chromosomes become bi-oriented, the kinetochore forces toward each pole cancel each other to a large extent, and the polar ejection force of the remaining chromokinesins is sufficient to allow for chromosome congression, explaining the observations in [5].

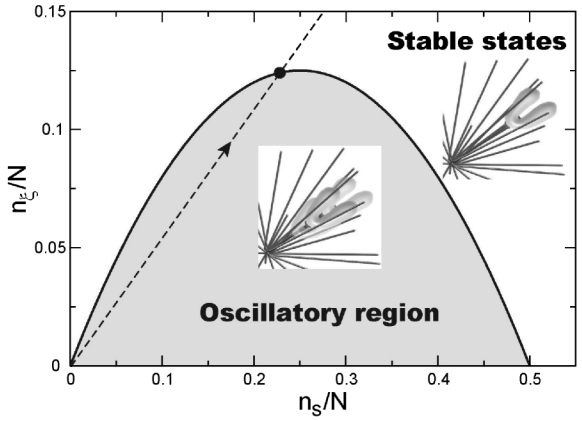

FIG. 2. Dynamical states of a mono-oriented chromosome ($f = 2$). Increasing n_s and n_ξ corresponds to increasing kinetochore force and chromosome friction, respectively. Decreasing the total chromokinesin number N (arrow) eventually leads to the disappearance of oscillations at $N = N_c$ (●).

Numerically computed chromosome motion in the unstable regime [Fig. 3(a)] displays the characteristic sawtooth-shaped oscillations observed *in vivo* [4] [Fig. 3(c)], indicating that the system switches suddenly between phases of constant velocities. Indeed, the period of the oscillation ($\sim$ min) is controlled by the forces on the chromosome, while the switching between phases occurs over much shorter time scales, characteristic of motor binding or unbinding ($\sim$ s). Close to the pole, the high density of MTs results in a large number of bound motors, driving the chromosome AP at a velocity close to their maximum velocity V_0 [Fig. 3(b)]. Thus, we argue that the velocity of the AP motion is a direct quantitative estimate of the chromokinesin velocity at vanishing load ($V_0 \simeq 2\ \mu$m/ min in Newt lung cells [3,4]). As the chromosome moves away from the pole, the density of MTs decreases and eventually reaches a value at which the attachment flux is too low to compensate the motor detachment. The remaining motors then detach rapidly [Fig. 3(b)] and the chromosome switches to P movement. The P phase occurs with almost no motors attached and the chromosome moves toward the pole with a constant velocity $-F_K/\xi$. The cycle is completed when the chromosome reaches a region of high enough MT density, where many motors abruptly attach and eject the chromosome. The ratio of AP and P velocities, approximately given by n_ξ/n_s, characterizes the symmetry of the oscillations. Symmetric oscillations are obtained for $n_\xi \simeq n_s$ and $f > 2$. In case $f < 2$, all states that fulfill $n_\xi \simeq n_s$ are stable and only asymmetric

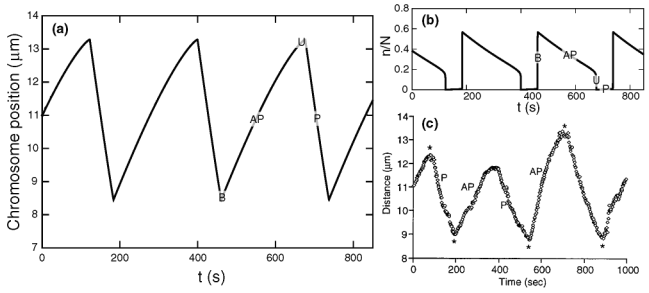

FIG. 3. Time evolution of the chromosome position (a) and the number of bound chromokinesins (b) obtained by numerical integration of Eq. (3). (c) Sawtooth-shaped chromosome oscillations observed *in vivo* (from Ref. [18]). Chromosome poleward (P) and away-from-the-pole (AP) phases are separated by dramatic chromokinesin binding (B) and unbinding (U) from MTs. The MT distribution is $k_b(r) = k_b^{(0)} S_{\rm ch} M/4\pi r^2$ (see text). The chromokinesin parameters are consistent with the experimental data: $V_0 = 2.38\ \mu$m/ min [4,12], $k_u^{(0)} = 1.65\ {\rm s}^{-1}$ (estimated from the chromokinesin processivity length $l_p \equiv V_0/k_u^0 \simeq 24$ nm [11]), and $f = 2$ [25]. The parameters $k_b(r) = 266/[r(\mu{\rm m})]^2\ {\rm s}^{-1}$, $n_s/N = 0.115$, and $n_\xi/N = 0.052$ are chosen to reproduce the amplitude, period, and average chromosome-to-pole distance of the *in vivo* oscillations in (c) [18]. The value of k_b is consistent with reasonable estimates for $M \simeq 500$, $S_{\rm ch} \simeq 10\ \mu{\rm m}^2$, and $k_b^{(0)} \simeq 1\ {\rm s}^{-1}$.

oscillations can be obtained. Figure 3 illustrates the possibility for asymmetric oscillations. The observation of symmetric oscillations [4,18] suggests that chromokinesins are highly sensitive to the applied force, in agreement with the observations in [12].

Similarly to chromokinesins, it is most likely that the force-producing entities responsible for the kinetochore force are themselves force sensitive. It has been argued that the kinetochore switches between a force-producing state in the P phase and a neutral state in the AP phase [6]. The mechanism proposed here for the AP force generation by chromokinesins can be extended to the force generation in the kinetochore, similarly to [7,13]. The existence of a neutral state can be naturally explained by the collective detachment of force generating entities in the kinetochore during AP motion, which occurs in the same way that chromokinesins detachment during P motion. We have checked that an extended framework including this effect leads to similar nonlinear oscillations. We emphasize that the essential ingredients for chromosome oscillations are the position-dependent chromokinesin attachment rate (providing spatial information via the MT density in the aster) and the collective force-dependent detachment kinetics of the motors, regardless whether the latter are chromokinesins, the elements responsible for the poleward force, or both.

We have extended the present analysis to the case of bioriented chromosomes by considering chromokinesin binding on a bipolar spindle composed of two microtubule asters [27]. Although the kinetochore forces cancel out to a large extent in this case, we find that the tug of war between the opposing polar ejection forces of the two MT asters may either stably position the chromosome at the mitotic plate or lead to sawtooth-shaped oscillations about it. We argue that Östergren's ''traction fiber model'' [28], stating that proper chromosome positioning requires the kinetochore force F_K to increase with the length of the MT fiber, should be understood in the more general sense. The net P force of each aster increases away from the pole because less chromokinesins are bound to the aster, resulting in a weaker polar ejection force. The symmetry of a bipolar spindle ensures that chromosomes correctly locate at the mitotic plate, where polar ejection forces from each pole balance. Indeed, it has been shown that chromokinesins are essential for proper chromosome alignment at the metaphase plate in several organisms [29].

In summary, we present a unifying framework in which chromosome movement, positioning, and congression can be explained from the same physical principles. We propose that chromokinesin binding onto MTs allows the chromosome to sense its position in space via the asterlike morphology of the MT spindle and that motor unbinding from MTs is the force-sensitive mechanism at the origin of the chromosome directional instability.

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