Chromatin compaction by condensin I, intra-kinetochore stretch and tension, and anaphase onset, in collective spindle assembly checkpoint interaction

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Received 26 November 2013
Accepted for publication 3 February 2014
Published 27 March 2014

Abstract

The control mechanism in mitosis and meiosis by which cells decide to inhibit or allow segregation, the so-called spindle assembly checkpoint (SAC), increases the fidelity of chromosome segregation. It acts like a clockwork mechanism which measures time in units of stable attachments of microtubules (MTs) to kinetochores (the order parameter). Stable MT–kinetochore attachments mediate poleward forces and ‘unstable’ attachments, acting alone or together with motor proteins on kinetochores via chromosomes, antipoleward forces. Stable and unstable attachments could be separated, and the non-equilibrium integrated MT mediated force acting on stably attached kinetochores was derived in a collective interaction (Matsson 2009 J. Phys.: Condens. Matter 21 502101), in which kinetochores were treated as rigid protein complexes. As forces and tension in that model became equally distributed in all bioriented sister chromatid (SC) pairs, segregation was inhibited without need of a ‘wait-anaphase’ signal. In this generalization, the kinetochore is divided into an inner chromatin proximal complex and an outer MT proximal complex, and the integrated MT mediated force is divided into an integrated poleward and an integrated antipoleward force. The model also describes the collective interaction of condensin I with chromatin, which together with the MT mediated dynamics yields the putative in vivo tension in kinetochores and centromeric and pericentromeric chromatin, as a non-linear function of the order parameter. Supported by the compaction force and an increased stiffness in chromatin towards the end of metaphase, the two opposing integrated MT mediated poleward forces, together with metaphase oscillations, induce a swift and synchronized anaphase onset by first increasing the intra-kinetochore stretch. This increase lowers the SAC energy threshold, making a cleavage by separase of all cohesin tethering SC pairs in anaphase energetically possible, thereby reducing the risk for aneuploidy and cancer. It is also shown how this risk might increase in condensin I depleted cells. Moreover, a solution is provided to the fundamental statistical physics problem with a system containing an increasing number of particles (molecular complexes) that become strongly correlated in space.

Keywords: spindle assembly checkpoint, chromatin compaction, condensin I, anaphase onset, intra-kinetochore stretch and tension

(Some figures may appear in colour only in the online journal)
1. Introduction

To increase the fidelity in chromosome segregation, the spindle assembly checkpoint (SAC) machinery must first ensure that all sister chromatid (SC) pairs have been (bioriented) stably attached at their kinetochores by microtubules (MT) from the two spindle poles [1–8]. Furthermore, to allow the anaphase onset, in higher eukaryotes the SC pairs must be positioned (aligned) at the metaphase plate equidistantly from the two poles (figure 1(a)), [4, 6, 8]. Stably attached kinetochores are hence strongly spatially correlated and the forces acting on them are position dependent [8–11]. This implies that MTs and chromosomes in the stably connected part of the system act together as one macromolecular unit. Defects in this machinery can lead to aneuploidy and cancer [12, 13].

In contrast to higher eukaryotes, in which spatial correlations between bioriented SC pairs at the metaphase plate are 'directly' observable (figure 1(a)), in budding yeast (Saccharomyces cerevisiae) bioriented SC pairs are spread out about the mother-bud axis (figure 1(b)) and sister kinetochores, more stretched apart (figure 2) at anaphase onset [14]. But the synchronization of the segregation process shows that kinetochores must be strongly correlated in space also in that case. The kinetochore is a complex of more than 80 proteins that connect MTs to centromeric and pericentromeric DNA [15]. However, in budding yeast each kinetochore is stably attached by one MT only (figure 2), making the structure of this spindle a bit less complex than that of higher eukaryotes [7].

The final separation of SC pairs is mediated by the anaphase promoting complex (APC) once it becomes activated by the protein Cdc20 [16–18]. Before that, unattached kinetochores recruit Mad and Bub proteins, which bind Cdc20 into the mitotic checkpoint complex (MCC), explaining the inhibition of anaphase at a molecular level [2, 5, 12, 19–24]. However, despite extensive studies, it has not been possible to describe how events at kinetochores become integrated to inhibit segregation until all SC pairs have been bioriented [24].

It is thought that SAC monitors the kinetochore–spindle interaction [7], suggesting that MT plus-ends and vacant kinetochores are the key reactants in this machinery. That was assumed already in the previous version of the collective model interaction [25], in which the kinetochore was treated as a rigid complex of proteins, and the compaction of chromatin was simply taken for granted.

Once an SC pair becomes bioriented, the two sister kinetochores are pulled apart by poleward MT mediated forces (figure 2), creating stretch and tension in pericentromeric chromatin and kinetochores [26–28]. Tension is balanced by cohesive forces mediated by cohesin that tethers the SCs pairwise together [29, 30] and by MT mediated antipoleward forces generated by motor proteins that act on kinetochores via the chromosomes [11]. All MT attachments that induce antipoleward forces are henceforth referred to as unstable. There is also a weak antipoleward compaction force (≤ 0.4 pN) generated by condensin I [31], which is loaded on chromatin in prometaphase and metaphase under release of cohesin [32] and turnover of histones [33]. However, a stretching force of 10 pN is needed to disrupt compacted chromatin [31], which can thus withstand the 5 pN thought to be required for segregation [33].

The generation and properties of forces are studied here in a generalized collective model interaction, in which SAC becomes swiftly released when chromatin is sufficiently compacted and kinetochores are maximally stretched in metaphase. As will be shown, the increase in intra-kinetochore stretch lowers the energy threshold to anaphase and thus makes a proteolytic separation of all SC pairs energetically possible. The system then unleashes Cdc20 from MCC for the activation of APC [18]. As in the previous version of the collective interaction [25], this generalization of the model is derived from three ‘first’ principles in which dissipative forces and the waggling of chromosomes are included: (1) a rate equation in which attachment and detachment are separated, (2) the initial boundary constraints for the two key reactants, and (3) a non-equilibrium formulation of the strong spatial correlations between stably attached kinetochores. These principles,
Apart from the key reactants, segregation depends on a myriad of factors, which seem to be 'enslaved' by the collective MT–kinetochore interaction in the sense that they are indispensable prerequisites for the three first principles. For instance, motor proteins generate the interpolar MT mediated forces that balance the collective forces required for kinetochores to become strongly correlated in space [11]. Changes in the turnover of MTs regulated by Aurora B/Ipl1, Ran, CLASP and CENP-E [40] are weighted into the on- and off-rate constants of the attachment rate equation. After all, a model of the SAC machinery would not be very realistic without a rate equation that separates on and off rates and drives the cell cycle forward, without boundary constraints for the key reactants that make a ‘book-keeping’ and counting of events possible, and without spatial correlations of stably attached kinetochores that yield position-dependent forces. Such a system could not progress through the cell cycle, distinguish stable from unstable attachments, count the number of stable attachments, nor know when the APC activity should be inhibited or allowed.

In the model presented here, anaphase onset is delayed until it becomes energetically possible. This transition is closely linked to the higher order folding and compaction of chromatin [32] and the increase in intra-kinetochore stretch [26–28]. As will be demonstrated, the collective interactions of cohesin, condensin I and histones with chromatin (and of separate with cohesin in anaphase), are formally equal to the collective MT–kinetochore interaction. Like the previous version of the model [25], this generalization is obtained for an idealized spindle–chromosome system, in which all sister kinetochore pairs are first equally stretched a distance 2D apart (figure 2) and then assembled at the centre of mass \( x_{\text{cm}} \) (figure 1(b)) on the interpolar axis. The \textit{in vivo} deviations from \( x_{\text{cm}} \) are then "fully" corrected for by the radius of gyration \( [41, 43] \), which is derived as in a freely jointed chain (FJC) [42].

After this correction, the collective interaction should provide a realistic model of the \textit{in vivo} SAC dynamics and the generation of intra- and inter-kinetochore tensions in higher eukaryotes and budding yeast. This may be verified indirectly by single molecule force-extension experiments \textit{in vitro}, but only for small stretches [44]. After the inclusion of a second-order expansion of the radius of gyration correction, the force-extension formula derived from the collective model interaction agrees with that obtained from single molecule stretching data [45]. Accordingly, together with the full radius of gyration factor, which accounts for an arbitrary spread of stably attached kinetochores in the spindle volume of a living cell, the integrated MT, cohesin, condensin I and histone mediated forces should yield the \textit{in vivo} tension in kinetochores and chromatin.

2. Rate of attachment and initial boundary constraints

Chromosome segregation depends on a large structural reorganization of chromatin. As already mentioned, these changes are mediated by cohesin and condensins, which contain the structural maintenance of chromatin (SMC) proteins [30, 32] and a large turnover of histones [46]. Cohesin establishes SC cohesion
already in S phase [29, 30], and is non-proteolytically removed in prophase, prometaphase and metaphase concomitantly with the loading of condensins [32]. Whereas condensin II folds chromatin already in prophase, condensin I gains access to and induces a higher order folding and compaction of chromatin only after nuclear envelope breakdown [32]. It is not understood exactly how condensin I interacts with chromatin (figure 3). However, in the presence of hydrolysable ATP, condensin I compacts single DNA molecules against a weak force of 0.4 pN and induces positive super-helical tension into ssDNA, possibly by forming loops [31, 32, 48–52]. What is clear is that condensin I is a major regulator of the stiffness of chromatin [53].

A complete description of the higher order folding of chromatin by condensin I in prometaphase and metaphase is beyond the scope of this article. But for a collective interaction, it suffices to consider condensin I as a ligand (dark green) in which the topological links are contracted (clustered) to one point, it suffices to consider condensin I as a ligand (dark green) with two binding sites.

where \( a = (\rho_0 + \sigma_0 + K)/2 \), \( K = k'lk, b' = \rho_0\sigma_0 \) and \( g^2 = (a^2 - b^2)/a^2 \). Although the parameter \( \mu \) is redundant here, it turns out to be useful later. The solution to equation (3a) reads

\[
\ln \left( \frac{\rho(x, t)}{\rho(x, t)' \rho_K} \right) = k(\rho_K - \sigma_K)(t - t_0) \equiv 2kag(t - t_0),
\]

At the same time as the fast turnover of unstable MT attachments boosts the spindle assembly, it also ‘screens’ vacant kinetochores, hence \( \sigma_K \leq \sigma_0 \). However, regardless of whether \( k' \neq 0 \) or not, the form of binding rate of the different pairs of reactants remains the same as in equations (3a) and (3b). When \( k' = 0 \), the attachment is more efficient and the screening of vacant kinetochores vanishes. The dynamic reactant concentrations \( \rho_K \), \( \sigma_K \), \( \rho' \) and \( \sigma' \) then become the usual ‘bare’ concentrations \( \rho_0, \sigma_0, \rho, \sigma \) (appendix), and equation (4) then yields the solution corresponding to stable bindings only.

As the concentration of MT plus-ends is much larger than that of kinetochores, it follows that \( \rho_K \gg \sigma_K \), and \( g = (\rho_K - \sigma_K)/(\rho_K + \sigma_K) = 1 \). Whether the same logic applies to the concentrations of cohesin, condensin I, histones and separase, and their binding sites on chromatin, is not known. However, as \( g \) will play a role as coupling constant in the collective spindle–SC interaction, it is anyway kept different from one. The binding sites for cohesin, condensin I, histones and separase on a particular chromosome are obviously spatially correlated to the corresponding kinetochore. But since all stably attached kinetochores become spatially correlated in prometaphase, the binding sites on all chromosomes of all such reactants become strongly correlated in space. This shortcoming therefore does not essentially change the form of the collective interaction.
3. Strong spatial correlations at non-equilibrium conditions

In higher eukaryotes, all bioriented SC pairs must be aligned on the metaphase plate before commitment to anaphase. However, the exact position on this plate seems to be less important. For simplicity, in the idealized model, all stably attached kinetochores are first contracted to the centre of mass \( x = (0, 0, 0) \), which is here assumed to be located where the metaphase plate cuts the interpolar axis (figure 1). The error committed by this approximation, which is larger in budding yeast than in higher eukaryotes, is then corrected for by the radius of gyration \([41, 43]\). To demonstrate how the binding sites for cohesin, condensin I, histones and separase on chromatin become spatially correlated, the spatial correlations between stably attached kinetochores are formulated first. But since the number of such kinetochores increases with time, a non-equilibrium probability theory is required.

A box with 33 pink and 67 white golf balls can serve to elucidate the statistical problem to formulate the spatial correlations that control the SAC dynamics. The probability of repeatedly picking out a pink ball from the box is 33% if the balls are each time replaced into the box. The probability density is in turn given by the concentration of pink balls \( \psi(x) \). Integrated over the box volume this yields a total probability \( \int \psi(x) \, dx = 33 \). Thus \( \psi(x) \) can be renormalized (divided by 33) such that \( \int \psi(x_1) \, dx_1 = 1 \), wherein \( \psi(x_1) \) represents the probability density to find one pink ball at \( x_1 \), \( \psi(x_1)\psi(x_2) \) the joint probability density to find one pink ball at \( x_1 \) and a second at \( x_2 \), and so on. At equilibrium conditions, the probability density to find the 2N stably attached kinetochores distributed at \( x_1, x_2, x_3, \ldots, x_{2N} \) becomes \( \psi(x_1)\psi(x_2)\ldots\psi(x_{2N}) \), as in the FJC model \([42]\). However, if the number of pink balls is increasing, \( \psi(x, t) \) depends on time and is therefore not normalizable. This is what happens in a living cell; SAC is on only as long as there are unattached kinetochores. The inhibiting mechanism thus works in a system with an increasing indefinite number of stable and unstable attachments that are indistinguishable at the molecular level, and which can be spread over an indefinite number of sites, \( x_1, x_2, x_3, \ldots, x_{n-1}, x_n, x_{n+1}, \ldots \), by an indefinite probability distribution

\[
p \{ \psi(x, t) \} \approx \sum_{i=1}^{\infty} C_i \prod_{j=1}^{\nu} \psi(x_j, t) . \tag{5}
\]

To account approximately for the ‘alignment’ on the metaphase plate, all stably attached kinetochores are contracted to the centre of mass \( x = (0, 0, 0) \). Equation (5) then becomes a geometric series in \( \psi(x, t)/a \), because equations (3a) and (3b) require that \( C_i = a^{-i} \). The errors committed by contracting all \( x_j \) to \( x \), disregarding the spread of stably attached kinetochores on and about the metaphase plate in higher eukaryotes (figure 1(a)) and about the mother-bud axis in budding yeast (figure 1(b)), are corrected for by the radius of gyration \([41, 43]\).

However, as the turnover of unstable attachments is more rapid than that of stable attachments, unstable attachments should attain a stationary state, \( d\psi_0(x, t)/dt = 0 \), before kinetochores start to become stably attached. Thus there should be a time-independent zero-order term \( \psi_0(x, t) \) in equation (5) that can also be factored out from all higher order terms. The joint probability density \( p(x, t) \) of having simultaneously a constant concentration of unstable attachments and an indefinite number of stable attachments at time \( t \) in the spindle volume can thus be written as \( p(x, t) = \psi_0(x, t) + \psi_1(x, t) \Sigma(\psi(x, t)/a)^2 \). Integrated over the spindle volume \( V \), with \( \mu = N\psi_0(x, t) \), the total probability \( \phi(x, t) = \mu \Sigma(\psi(x, t)/a)^2 \) then becomes

\[
\psi(x, t) = \frac{1}{\mu - \psi(x, t)/a}. \tag{6}
\]

The contraction to \( x = 0 \) implies that the time-dependent (non-equilibrium) concentration of stably attached kinetochores becomes constant in space, \( \psi(x, t) = \psi(x, t) \), and the collective attachment via \( \psi(x, t) \) in equation (6) cannot be separated from that of stable attachments until after normalization of \( \phi(x, t) = \phi(t, x) \), whereby the number of stable attachments is determined.

The higher order folding of chromatin and the proteolytic separation of SCs are described similarly by contracting all binding sites on chromosomes for cohesin, condensin I, histones and separase to their respective kinetochores. Given that all stably attached kinetochores become strongly correlated in space, it follows that all binding sites of cohesin, condensin I, histones and separase on all chromosomes become strongly correlated in space. Therefore, since the first principles for these reactants are equal to those of the MT mediated dynamics, the corresponding collective interactions are formally equal too.

In order to obtain the time evolution of the SAC machinery, equation (6) must be combined with equation (3b). As the time derivative of equation (6) is given by

\[
\frac{d\phi(x, t)}{dt} = \frac{\mu}{a} \frac{1}{1 - \psi(x, t)/a^2} \frac{d\psi(x, t)}{dt}, \tag{7}
\]

the dependence on time of the collective SAC dynamics is obtained by insertion of equation (3b) into equation (7),

\[
\frac{d\phi(x, t)}{dt} = \frac{k\mu}{a} \left( \frac{\mu^2 - g^2 \phi(x, t)^2}{\mu^2-g^2} \right), \tag{8}
\]

which has the solutions

\[
\phi(t) = \frac{\pm \mu}{g}, \phi(t) = \pm \frac{\mu}{g} \tanh(kag(t-t_0)). \tag{9}
\]

To ensure that biorientation is completed, chromatin is sufficiently compacted and all SC pairs are separated, (1) the \( 2N \) kinetochores must be exposed to the MT plus-ends, (2) the \( 2NaN \) binding sites on chromatin must be exposed to condensin I and (3) the \( 2p/N \) cohesin molecules tethering SC pairs in anaphase must be exposed to separase, for a sufficiently long time: \( t_{a0} \gg t_1 = 1/k_{a/a}, i = 1, 2, 3 \) denoting the respective reactants. The turnover of histones follows the same first principles and dynamics but will be discussed here later. Of the \( 2\gamma N \) cohesin molecules that tether the NSC pairs together in S phase,
non-proteolytically removed before anaphase onset. The probability \( \phi(t) \) is then normalized by a so-called topological quantization \([54]\), \( 2 \phi N = \phi(t_0) - \phi(-t_0) = 2/\xi \), where \( \phi_1 = 1 \), \( \phi_2 = \alpha \) and \( \phi_3 = \beta \), and whereby the zero-order constant term \( \alpha = \frac{\mu}{\rho}(x_{cm}) \) representing unstable attachments in equation (6) is automatically subtracted out. But the question is now: does the ‘unsubtracted’ \( \phi(t) \) yields the number of stable attachments?

4. Collective spindle checkpoint dynamics

After contraction of all \( x_i \) in equation (5) to the centre of mass \( x_{cm} \) (or an arbitrary point \( x \) in the spindle volume), the idealized collective model the structure of the system and the order in which kinetochores are attached are unimportant. Thus the time-dependent solution in equation (9) can be interpreted as a travelling wave in a one-dimensional lattice of length \( L \) with spacing \( \Delta = L/2N \), where calculations are carried out in the continuum approximation \((N \to \infty)\) \([55]\). The errors committed by this approximation are corrected for by the radius of gyration \([41, 43]\). Multiplied by \( 1/v \), where \( v = \Delta a \) is the velocity of the travelling wave and \( x(t) = vt \) its position, equation (8) with \( \phi(t)/N \) replaced by \( \xi(t) \) then yields the travelling wave equation

\[
\frac{d\xi(x, t)}{dt} = \xi(1 - \xi(x, t)^2),
\]

which has the solutions \( \xi(x, t) = \pm 1 \) and

\[
\xi(x(t)) = \pm \tanh\left(\frac{g\chi(t)}{\Delta}\right).
\]

After multiplication by \( D^2 \), the elastic modulus \( \epsilon \) and the cross-section area \( A \) of the spindle fibres, the square of equation (10) yields the potential energy of the system, \( V_0(\xi) = D^2\epsilon A g^2(1 - \xi^2)^2/(2\Delta^2) = D^2\epsilon A (d\xi/dx)^2/2 \) \([54]\), in which \( \xi(x, t) \) represents the extension \( \chi \) per unit length \( D \) of the lattice (see the appendix). The derivative of \( V_0(\xi) \) gives the dispersive part of the equation of motion, \( dV_0/d\xi^2 = D^2\epsilon A d^2\xi^2/dx^2 \), showing that \( V_0(\xi) \) actually plays the role as collective potential energy of the stably attached kinetochore lattice in the continuum approximation \([54, 55]\). By addition of the second-order time derivative, which corresponds to acceleration in Newton’s force law, the equation of motion reads

\[
D^2\eta \frac{d^2\xi}{dt^2} - D^2\epsilon A \frac{d\xi}{dx^2} = -\frac{dV_0}{d\xi},
\]

where \( \eta = Ap \) is the linear mass density, \( \rho = \epsilon/\Delta^2 \) is the mass density of the lattice in the continuous approximation and \( s \) is the velocity for propagation of lattice oscillations.

However, for this dynamics to become realistic, two unphysical features must be removed. The travelling wave solution \( \phi(t) = N\tanh(ka\tau) \), representing the probability of finding stably attached kinetochores in the spindle volume, is negative for \( t < 0 \). Moreover, the potential energy has a local (unstable) maximum at \( \xi = 0 \), \( V_0(0) = D^2\epsilon A g^2(2\Delta^2) > 0 \), in the absence of stable attachments (excitations). Both of these flaws are corrected by a shift in the order parameter, \( \xi \to -1 + (\phi \to -N + \phi) \), which makes the probability \( \phi(t) \) positive definite for all times \((0 \leq \phi(t) \leq 2N)\).

\[
\phi(t) = N[1 + \tanh(ka\tau - t_0)].
\]

After this change of the constant term in equation (6), \( \xi(t) = \phi(t)/N \) represents the increasing number of stable attachments only and the shifted (asymmetric) collective potential energy \( V_\delta(\xi) = D^2\epsilon A g^2\xi^2(2 - \xi^2)/(2\Delta^2) \) vanishes in the absence of stable attachments, \( V_\delta(0) = 0 \).

When the order parameter is shifted, equation (12) becomes

\[
D^2\eta \frac{d^2\xi}{dt^2} - D^2\epsilon A \frac{d\xi}{dx^2} = -\frac{dV_\delta(\xi)}{d\xi} \equiv F(\xi)
\]

\[= -\frac{k}{2} \xi(1 - \xi)(2 - \xi),\]

where \( k = 4\epsilon A^2 g^2 = k_0G^2 = Dk_0 = Dk_0G^2 \) is the ‘generalized’ effective spring constant, \( k_0 \) is the effective spring constant, \( G^2 = (D/\Delta)^2 \) is the radius of gyration and stretch factor (to be determined), and \( k_0 \) and \( k_0G^2 \) are the generalized and the usual Hooke’s spring constants. Equation (14) resembles the equation of motion of a rigid rod (see the appendix) with cross-section area \( A \), or a string with elastic support \([25, 56]\). This equation can also be written as

\[
Dn \frac{d\chi}{dt} - D\epsilon A \frac{d\chi}{dx} = -\frac{dV(\chi)}{d\chi} \equiv F(\chi)
\]

\[[= -\frac{\chi}{2} \left(1 - \frac{\chi}{D} \right) \left(2 - \frac{\chi}{D} \right),\]

where \( \chi(t) = \xi(t)/D \) is the stretch parameter, \( V(\chi) = k_0 \chi^2 (2 - \chi/D)^2/8 = DV_\delta(\xi) \) is the collective potential energy (here with the ‘correct’ dimension), and \( F(\chi/D) = F(\xi) \) is the MT mediated integrated (collective) driving force that acts equally on all stably attached kinetochores. Dissipative forces and the waggling of chromosomes are effects accounted for in the average dynamics via the three first principles, may well renormalize the effective mass \( Dn \), but equation (15), which yields the dynamics of the stably connected part of the system, will still contain second-order derivatives in time and space. Like in equilibrium systems, \( F(\xi) \) could thus be obtained from the derivative of the potential energy.

Conversely, a direct summation of different force components would not be possible due to lack of knowledge of all force generators, the non-linearity implied by strong spatial correlations between stably attached kinetochores, and the problem of distinguishing stable from unstable attachments. The question was instead reduced to determining \( V(\xi) \) from first principles: (1) the rate equation for the key reactants, (2) the initial boundary constraints of the reactant concentrations, and (3) the strong spatial correlations between MT attachments. To be able to make the coherent approximation required to obtain equation (6) from equation (5) and then \( V(\xi) \), it has also been assumed that condensin II has induced a sufficient stiffness in chromatin already in prophase, after which condensin I induces the higher order folding in prometaphase and metaphase \([32]\).
With fully compacted chromatin at the end of metaphase, the integrated poleward and antipoleward forces, combined with metaphase oscillations, then finally increase the intra-kinetochore stretch and promote a swift anaphase onset \[26, 27\]. To study the stretch and the tension in and between kinetochores, which are here divided into one inner chromatin proximal protein complex and one outer MT proximal complex, the integrated MT mediated force \( F(\xi) \) is divided into one integrated poleward force \( F_p(\xi) \) and one integrated antipoleward force \( F_a(\xi) \). The compaction force generated by condensin I is then added separately.

### 5. New kind of intra-kinetochore tension generation

The integrated MT mediated force \( F(\xi) \) derived from the collective potential energy is skew-symmetric about \( \xi = 1 \), with a poleward peak of 5 pN at \( \xi = 1.577 \) and an equal antipoleward peak at \( \xi = 0.423 \) (figure 4(a)). This suggests that the integrated poleward and antipoleward forces \( F_p(\xi) \) and \( F_a(\xi) \) are skew-symmetric too, hence \( F_p(\xi) = -F_a(2 - \xi) \) (see the appendix). In fact, the sum of \( F_p(q, \xi) = -\kappa_0 G^2 \xi (2 - \xi) \) yields the actual force \( F(\xi) = -\kappa_0 G^2 \xi (1 - \xi)(2 - \xi)/2 = -\kappa_0 G^2 (\xi - 3\xi^2/2 + \xi^3)/2 \) (figure 4(a)). The most skew-symmetric case is obtained for \( q = 2.001 \approx 2 \). With \( G^2 = 1 \) and \( \kappa_0 = 1 \) pN, the peak of \( F(\xi) \) at \( \xi = 1.577 \) becomes 0.192 pN, and for \( \kappa_0 = 5/0.192 = 26 \) pN, \( F(\xi)_{\text{max}} \) increases to 5 pN, which is the force thought to be required for segregation [33]. The corresponding opposing forces, with \( F_p(q, \xi)_{\text{max}} = -F_a(q, \xi)_{\text{min}} = 7.704 \) pN at \( \xi = 1.333 \) and \( \xi = 0.667 \) (figure 4(a)), are then still safely below the 10 pN at which chromatin is disrupted [31].

However, since chromosomes are segregated by the two integrated poleward forces, it suffices that \( F_p(q, \xi)_{\text{max}} = -F_a(q, \xi)_{\text{min}} = 5 \) pN, and \( F(\xi) \) can be allowed to become arbitrarily small. The question is then reduced to determining the most realistic value of \( q \) and the strength of \( F(\xi) \). As will be shown, with no
restrictions other than $q > 2$, the tensions in kinetochores and chromatin become different. To make these tensions equal for all values of $ξ$, the compaction force must be added and the strength of the integrated MT mediated force reduced to the same strength as the compaction force, hence $F(ξ)_{\text{max}} = 0.4 \mu\text{N}$ [31]. These requirements are fulfilled when $q = 10$, $k_B(10) = 2.2 \mu\text{pN}$ and $G^2 = 1$ (figure 4(c)). However, the forces $F(ξ)$, $F_p(ξ, ξ)$ and $F_s(q, ξ)$, and hence also the tension, will become modified by the $ξ$-dependent radius of gyration and stretch factor $G(ξ, \theta)^2 = (2ξ(ξ/Δξ, \theta))^2$ (figure 4(b) and (d)), which is derived here later.

As shown in figure 4(a), in the first half of prometaphase ($0 < ξ < 1$), the integrated poleward force $F_p(ξ)$ is dominated by the integrated antipoleward force $F_s(ξ)$, which is induced by polar ejection forces and mediated by unstably attached MTs, centromeric and pericentromeric chromatin, and between the inner and outer kinetochore complexes, even at $ξ = 1$ where $F(ξ) = 0$. This tug-of-war continues in the second half of prometaphase ($1 < ξ < 2$), where the integrated poleward force $F_p(ξ)$ dominates the integrated antipoleward force $F_s(ξ)$ (figure 4(a)). Forces and tension are thus regulated in a highly nonequilibrium manner.

By depletion of condensin I or treatment with nocodazole, the intra-kinetochore stretch is suppressed and the APC activity is significantly delayed [27]. The compaction of chromatin by condensin I thus contributes significantly to the increase in intra-kinetochore stretch and the swift anaphase entry. As will be shown, the triggering of anaphase onset, which occurs when chromatin has become sufficiently compacted and kinetochores are maximally stretched in metaphase, is mechanically linked to a corresponding reduction of the energy gap between the oscillatory state [57, 58] and the so-called ‘branch-point’ of the oscillatory spectrum, by which chromosomes become synchronously separated [25, 54]. This reduction of energy gap corresponds to a lowering of the energy threshold to enter anaphase, which makes a proteolytic removal of all cohesin tethering SCs in anaphase energetically possible (figure 5). It will also be shown how the synchronization of segregation depends on the strong spatial correlations defined in equation (6).

6. Segregation preceded by intra- and inter-kinetochore oscillations

Equation (14) describes the collective interaction, which ensures that tension is distributed equally and kept non-zero in all bioriented SC pairs until the chromosomes become separated. Kinetochores thus remain stably attached and segregation inhibited until tension has relaxed, without need of a ‘wait-anaphase’ signal. However, in this model there is no lag time between the last stable attachment at $ξ = 2$ and the final chromosome separation, which also occurs at $ξ = 2$. This flaw is caused by the continuum approximation ($Δ = L/2N \rightarrow 0$; $N \rightarrow \infty$) in equation (5) [55]. But in reality, obviously there is such a lag time, because the stable attachments, the loading of condensin I in prometaphase [32], the transitions to the oscillatory states in metaphase [57] and anaphase [58], and to the threshold for chromosome separation [25, 54], are all stepwise events.

As shown in the previous model [25], the oscillatory spectrum is obtained by inserting a time-dependent perturbation $ξ(x, t) = ξ(x) + ξ(t)e^{iωt}$ in equation (12). For small $ξ(x)$, this yields an exactly solvable Schrödinger-like equation determined by the second derivative of the potential energy $-(d^2/dx^2 + V_{\text{G}}c_α(ξ,x)) ≡ -(d^2/dx^2 + 4ξ^2/Δ^2 - 6(4ξ^2/Δ^2)\cos^2(gξ/Δ))^2c_α(ξ,x) = (ω_0^2ξ^2c_α(ξ,x) [54]. This equation, with eigenfunctions $ξ_α(ξ)$, has a spectrum of discrete eigenfrequencies $f_n = ω_n/2\pi = (sg(2n\pi)/n(4 - n), n = 0, 1, and a continuum $ω_η^2 = (4 + (v^2/5))^2 s^2/Δ^2$ starting at the branch-point $f_0 = sg(\Delta n)$ where all SC pairs become synchronously separated, $v$ being the velocity of centromeres towards the spindle poles and $v_0 = (v^2/5) = (0.36 ± 0.16) \mu\text{m min}^{-1}$ their average velocity [58]. The collective potential energy, $V(ξ)$, thus controls its own oscillatory relaxation via its second derivative. This ensures that the energy required to transit from $f_0 = 0$ (the so-called translation mode) to $f_1 = sg(3/2\pi)$, and further to the branch-point $f_{b0} = sg(\Delta n)$ (which in the discrete spectrum corresponds to $n = 2$), is shared equally between all SC pairs, a necessary prerequisite for segregation to be synchronized.

The velocity $s$ is expected to be larger than $v_0$, and therefore the somewhat higher value $s = (0.39 ± 0.16) \mu\text{m min}^{-1}$ is used here [58]. Obviously, both $s$ and $Δ$ in chromatin will change with the release of cohesin and the loading of condensin I [32]. However, with maximally compacted chromatin at the end of metaphase, and with two-component kinetochores, the oscillatory spectrum should depend more on changes in the intra-kinetochore distance $δ$ (figure 2), than on the lattice spacing $Δ$, when kinetochores are treated as rigid point-like protein complexes. Thus with $Δ = δ_M = 0.065 \mu\text{m}$ [26] and $g = 1$, the metaphase frequency becomes $f_{1M} = (1.65 ± 0.68) \text{min}^{-1}$, which agrees well with that observed at $2.1 \text{min}^{-1}$ (~3.5 cycles/100 s) [57]. Similarly, with $Δ = δ_A = 0.100 \mu\text{m}$ [26], the frequency in anaphase becomes $f_{1A} = (1.08 ± 0.44) \text{min}^{-1}$, which agrees well with centromere oscillations observed at $f_{1A} = 1 \text{min}^{-1}$ [58]. With a lattice spacing $δ_A = 0.100 \mu\text{m}$, the branch-point for segregation corresponds to a frequency $f_{b0} = (1.24 ± 0.51) \text{min}^{-1}$.

By promoting the increase in intra-kinetochore stretch [26, 28], the continued compaction of chromatin by condensin I in metaphase [32] contributes to a swift anaphase onset and release of Cdc20 from MCC [18, 34, 35]. The branch-point at $f_{b0}$ can also be viewed as an attempted oscillation at $n = 2$, where SC pairs become instead synchronously separated. Without the strong spatial correlations implemented by Equation (6), there would be no collective oscillatory spectrum, because the $N$ SC pairs would then act like $N$ unsynchronized oscillators. Synchronization, and hence faithfulness, of the entire segregation process thus depend crucially on the spatial correlations between the kinetochores, and between the binding sites on chromatin for cohesin, condensin I, histones, separate and other enslaved factors.

7. Anaphase inhibited when chromosomes are sufficiently compact

Both the completion of biorientation and the alignment of SC pairs at metaphase plate in higher eukaryotes are mechanically linked to the anaphase onset. The SAC machinery thus
This increase in MA, which can be estimated from $E(\text{figure 5})$, corresponds to a lowering of the energy threshold for anaphase entry. This reduction of collective potential energy is in turn controlled by the oscillatory spectrum, which is determined by the second derivative of $V(\xi)$. However, an increase of the inter-kinetochore distance 2$D$, such as in $S. cerevisiae$ and condensin I depleted eukaryotic cells, may further reduce the collective potential energy and hence the threshold to enter anaphase, thereby increasing the risk of cancer.

Figure 5. The relaxation of collective potential energy at $\xi = \xi_\text{in} = 1.875$, from $V(\xi) = (\kappa/8)D[\delta_M^2(2 - \delta_M^2)/\delta_M^2 + \kappa M^2(2 - \delta_M^2)/\delta_M^2]$ in metaphase (blue curve) to $V(\xi) = (\kappa/8)D[\delta_M^2(2 - \delta_M^2)/\delta_M^2 + \kappa M^2(2 - \delta_M^2)/\delta_M^2]$ in anaphase (red curve), where $\delta_M = 65 \text{ nm}$ and $\delta_S = 100 \text{ nm}$ [26], corresponds to a lowering of the energy threshold for anaphase entry. However, to test if such a relationship exists for small eukaryotes, it follows that the rate of increase in spindle length should be given by equation (13),

$$\lambda(t) = \lambda_0[1 + \tanh((t - t_0)/\tau_3)]. \quad (16)$$

For $S. cerevisiae$, a topological quantization extended over 2$D_{30}$ = 6 min appears to be sufficient for the $2pN$ cohesin molecules tethering the $N$ SC pairs in anaphase to become synchronously cleaved by separase. With a time scale $\tau_3 = 1/(k_3A_{SG}) = 1$ at the molecular level, equation (16) fits the data well (figure 6) for this type of cell [60].

This yields the sum rule $2\pi_3 = (\rho_{30} + \sigma_{30} + k_3/\kappa_3) = 2(k_3\Lambda)$ between the on- and off-rate constants for separase and the initial concentrations of separase and bound cohesin, $\rho_{30}$ and $\sigma_{30} = 2pN/\Lambda$, where $\Lambda$ is the spindle volume and $2pN$ the number of cohesin molecules that holds the $N$ SC pairs together in anaphase. Curves similar to that in figure 6 should be obtainable also for e.g. Caenorhabditis elegans and Drosophila melanogaster [60].

9. DNA stretching by external force

That stretching of a DNA molecule in the laboratory by an external force [45] does not correspond to the in vivo dynamics of chromatin in a living cell has been clearly stated [44]. However, to test if such a relationship exists for small stretches, it is necessary to obtain the corrections to $D$ and $\lambda$ in the idealized collective model. In budding yeast the average...
lattice $\Delta$ should increase with the average stretch of the lattice along the mother-bud axis as $\Delta^2$. But since $F(\xi)$ in equation (14) is a result of the displacement, $\xi \rightarrow -1 + \xi$, $\Delta^2$ in the denominator of $\kappa = k_0 D^2/\Delta^2$ should be replaced by $\Delta^2(1 - \xi)^2$.

In the idealized collective model, all stably attached point-like and rigid kinetochore are stretched a distance $D$ perpendicular from the metaphase plate (figure 2). All kinetochores are then contracted to the centre of mass $x_m = (0, 0, 0)$. To correct for this, $D$ in the numerator of $\kappa = k_0 D^2/\Delta^2$ is replaced by the actual vectors $x_k$ from $x_m$ to the respective kinetochrome (figure 1(b)). After a displacement $-D \rightarrow D + x_k$, with $D = (D, 0, 0)$, which corresponds to $\xi \rightarrow -1 + \xi$, $D^2$ in the numerator of $\kappa$ becomes replaced by $(D + x_k)^2 = D^2 + x_k^2$, $\langle D - x_k \rangle = D x_m = 0$, $R_\parallel$ being the radius of gyration, $R_\perp^2 = L_m^2$ [41, 43], and $L_m^2 = (D\xi)^2$ the mean square end-to-end length of the idealized lattice along the polar axis, $D^2$ in the numerator of $\kappa$ thus becomes replaced by $D^2(1 + \xi^2)/6$. With these corrections, the generalized effective spring constant becomes $\kappa = k_0 D(\Delta/\xi)^2(1 + \xi^2/6)/(1 - \xi)^2$. If the stretches of pericentromeric chromatin in different SC pairs overlap each other, $\Delta$ in the denominator of $\kappa$ becomes replaced by an effective distance $\Delta_{eff}$, which with two-component kinetochore is replaced in turn replaced by the inter-kinetochrome distance $\delta$. The factor $k_0 D(\Delta/\xi)^2$ of the generalized effective spring constant is here set equal to $3k_BTb_k$, where $b_k$ denotes the Boltzmann constant, $T$ the temperature, $b_k$ the effective Kuhn segment length, and $p$ the persistence length [42]. For small stretches of pericentromeric chromatin ($\xi < 1$) the integrated force $F(\xi)$ acting on point-like rigid kinetochore 'in vivo', including the second-order correction from the radius of gyration factor, then becomes

$$F(\xi) = \frac{\kappa_0 D^2}{\Delta^2} (\xi(1 - \xi)(2 - \xi) + \xi^3/3)$$

$$= \frac{\kappa_0 D^2}{\Delta^2} \left(\xi + \frac{1}{4(1 - \xi)^2} - \frac{1}{4}\right).$$

(17)

This explains the relationship between the force-extension formula of a single DNA molecule [45], the worm-like chain (WLC) model [42,61], and the idealized collective MT mediated spindle–SC interaction defined by equation (14). The three first principles thus seem to well characterize the idealized SAC dynamics.

10. In vivo tension in the dividing cell

Accordingly, equation (17) yields the integrated MT mediated force for small stretches ($\xi < 1$) of pericentromeric chromatin in an idealized 'living' cell with rigid kinetochores. But equation (17) also holds for larger stretches in the study of a single DNA molecule in vitro [45]. To account for the actual spread of stably attached kinetochores in the spindle volume of a living cell (figure 1(b)) for all values of the order parameter, $0 < \xi < 2$, $F(\xi)$ must include the expanded full radius of gyration and stretch factor $(1 + \xi^2/6)(1 - \xi)^2$, which unfortunately diverges at $\xi = 1$. Obviously, this is not the case in three dimensions where stably attached kinetochores in bioriented SC pairs are spread an average distance $\theta\Omega$ perpendicularly from the mother-bud axis.

Therefore $\Delta^2$ should be replaced by $\Delta^2((1 - \xi)^2 + \theta^2)$ because intersecting MTs block a perfect alignment ($\theta = 0$) with the interpolar axis. The integrated MT mediated force acting on stably attached kinetochores in budding yeast should thus read

$$F(\xi) \approx -\frac{\kappa_0 D^2(1 + \xi^2/6)}{2\Delta^2((1 - \xi)^2 + \theta^2)} \xi(1 - \xi)(2 - \xi).$$

(18)

In higher eukaryotes, stably attached kinetochores are spread on and about the metaphase plate and the lattice is then essentially perpendicular to the polar axis, making $D$ and $\Delta$ independent of $\xi$. In that case, $F(\xi)$ attains the same non-linear form as in equation (14).

With $G(\xi, 0)$, $\theta^2 = (D(\xi)/\Delta(\xi, 0))^2$, the general formulation of $F(\xi)$ should read

$$F(\xi) = -\frac{\kappa_0 D^2}{\Delta^2(\xi, \theta)^2} \xi(1 - \xi)(2 - \xi),$$

(19)

where $D(\xi)^2 = D^2(1 + \xi^2/6)$ and $\Delta(\xi, \theta)^2 = \Delta^2((1 - \xi)^2 + \theta^2)$ in budding yeast (probably also in condensin I depleted higher eukaryotic cells), and $D(\xi)/\Delta(\xi, \theta) = D_{eff}/\Delta_{eff}$ in higher eukaryotes. A model in three spatial dimensions could perhaps predict $F(\xi)$ better, but the mathematics would become much more complicated [62].

The intra-kinetochrome tension is defined by the difference between the two integrated MT mediated force components,

$$T_i^\xi(q, \xi) = \frac{1}{2}(F_i(q, \xi) - F_i(q, 0))$$

$$= \frac{\kappa_0 G(\xi, \theta)^2}{4} (2 - \xi)(q - 1).$$

(20)

Thus whereas $F(\xi)$ vanishes at $\xi = 1$, this is not the case for the intra-kinetochrome tension, $T_i^\xi(q, 1) = (F_i(q,1) - F_i(q,1))/2 = \kappa_0 G(1, \theta)^2(q - 1)/4$, which becomes clear also from figure 4(c).

The inter-kinetochrome tension in centromeric and pericentromeric chromatin $T_c^\xi(q, \xi)$ should be equal but opposite to the integrated MT mediated antipoleward force $F_i(q, \xi)$.

$$T_c^\xi(q, \xi) = -F_i(q, \xi) = \frac{\kappa_0 G(\xi, \theta)^2}{4} (2 - \xi)(q - 1),$$

(21)

which, like $T_i^\xi(q, \xi)$, vanishes at $\xi = 0$ (without attachments there is no tension). $T_i(q, \xi) and T_c(q, \xi)$ also seem to vanish at $\xi = 2$, which may first look correct because the viscou drag does not act after until chromosome separation. But the tension generated after $\xi_{th} \leq 1.875$ has not yet relaxed at the end of prometaphase [59]. This flaw is expected though, because in the spatially coherent (collective) dynamics, which correspond to the continuum approximation of the discrete kinetochrome ‘lattice’ [55], both metaphase and anaphase erroneously seem to take place instantly at end of prometaphase ($\xi = 2$).

Unfortunately, the tension in kinetochores (equation (20)) differs from the tension in chromosome (equation (21)), $T_i(q, \xi) \neq T_c^\xi(q, \xi)$. However, this too is expected because $F(\xi) \equiv F_{MT}(\xi)$ accounts only for the integrated MT mediated force but not for the compaction force $F_{com}(\xi)$. But since $F_{com}(\xi)$ should have the same form as $F_{MT}(\xi)$, the question reduces to determining $q$ and the strength of $F_{MT}(\xi) = F_i(q, \xi) + F_c(q, \xi)$ (see the appendix). Since metaphase oscillations can
start only when poleward and antipoleward forces balance each other, \( F_{\text{MT}}(\xi) \) should have the same strength as \( F_{\text{conf}}(\xi) \), hence \( F_{\text{MT}}(\xi)_{\text{max}} = 0.4 \text{pN} \). Moreover, as tension is generated by the two opposite integrated MT mediated forces \( F_{\text{MT}}(q, \xi) \) directed towards the spindle poles (figure 1), it suffices that in vivo caused by MT attachments, the putative turmoil from recent attachments or detachments of MTs or other, particularly obvious in metaphase, where MT attachments relax as a function of the order \( \xi \) of various proteins, tension should be constant from pole to pole. The putative \( F_{\text{MT}}(\xi) \) as a function of the order \( \xi \) equals \( -F_{\text{MT}}(q, \xi) \), hence

\[
T_i(10, \xi) = \frac{1}{2} (F_{\text{MT}}(q, \xi) - F_{\text{MT}}(q, \xi) + F_{\text{MT}}(\xi)) = F_{\text{MT}}(10, \xi). \quad (22)
\]

with \( q = 10 \) and \( k_0(10) = 2.2 \text{pN} \), the tension in chromatin thus becomes equal to the tension in kinetics, \( T_i(10, \xi) = T_i(10, \xi) = F_{\text{MT}}(10, \xi) \). Conversely, this proves that \( F_{\text{MT}}(\xi) \) actually equals \( -F_{\text{MT}}(\xi) \) because, except for intermissions of turmoil from recent attachments or detachments of MTs or various proteins, tension should be constant from pole to pole. The putative in vivo tension \( T_i(\xi) \) as a function of the order parameter should thus read

\[
T_i(\xi) = F_{\text{MT}}(10, \xi) = \frac{k_0}{2} G(\xi, \theta)^2 \xi^2 (2 - \xi)(\xi + 8), \quad (24)
\]

which for \( G(\xi, \theta)^2 = 1 \) attains the maximum 5 \text{pN} at \( \xi = 1.055 \) (figure 4(e)). The in vivo tension at the end of prometaphase should then become \( T_i(\xi_{\text{th}}) = F_{\text{MT}}(10, \xi_{\text{th}}) \).

11. Collective control of APC

At molecular level, the activation of APC by Cdc20 is inhibited by the checkpoint proteins Mad2 and BubR1 [8, 12, 18, 34–36]. This locally acting inhibition mechanism is, in turn, subjected to a collective (global) control linked to the spindle assembly process. It may appear as if the last stable attachment triggers anaphase onset, but it is not that simple. There are many complex intermediate steps. Earlier stably attached chromosomes, or bioriented SC pairs, first control if the last arriving MT attachment is unstable or stable. In the latter case, this implies that the collective potential energy, and hence forces and tension, become redistributed and equalized at new levels. This relaxation of energy is controlled by the collective potential energy \( V(\xi) \) itself and its second derivative via the oscillatory spectrum. In higher eukaryotes, this equalization process is preceded by the alignment of all bioriented SC pairs on the metaphase plate.

At molecular level, the equalization of forces and tension is a complex process that may depend on many different factors such as MT flux [59]. However, in the collective dynamics, the equalization of energy is a result of the spatial coherence, which was implemented by volume-averaged concentrations of the stably attached kinetochores. Spatial coherence prevails also in budding yeast, although there is no alignment.

In metaphase, the relaxation of the collective potential energy proceeds via two discrete eigenstates, an oscillatory mode and a branch-point, and at the end of this phase the intra-kinetochore distance increases from about 65 to 100 nm. This induces a collective and synchronized anaphase onset, by lowering of the energy threshold to anaphase and by instantly changing the two eigenstates in metaphase into the oscillation and the branch-point in anaphase. Thus, whereas in metaphase the energy consumed for the transition from the oscillatory state to the branch-point is just sufficient to increase the intra-kinetochore stretch from ~65 to ~100 nm, in anaphase the energy difference between these two states is sufficient to proteolytically remove all cohesion in anaphase, i.e. for a synchronized and ordered separation of all replicated chromosomes. As was shown above, this quantal amount of energy could be estimated by equating the lowering of collective potential energy with the decrease in energy difference between the oscillatory mode and the branch-point when \( \delta \) increases from 65 to 100 nm. It may be speculated that this decrease in collective potential energy at the molecular level corresponds to the APC dependent ubiquitination–deubiquitination activity of Cdc20 and other SAC proteins, by which Cdc20 becomes released from MCC [34, 35].

As for the MT–kinetochore attachment, the association of Cdc20 with Mad and Bub proteins into MCC can be described by equation (1). The formation of MCC is promoted by unattached kinetochores [12, 24, 34], to which it becomes spatially correlated. Individual MCC complexes then become correlated to each other when biorientation progresses, implying that the collective APC–Cdc20 interaction [18] is formally equal to (enslaved by) the collective MT–kinetochore dynamics too. This provides a second aspect on the mechanism by which anaphase onset is synchronized and collectively linked to the completion of biorientation (alignment in higher eukaryotes), the first aspect being the lowering of the energy threshold to anaphase when \( \delta \) increases. However, it remains to be shown how the release of Cdc20 from MCC is linked to the increase in intra-kinetochore stretch at the molecular level.

12. Discussion and outlook

Despite the progress in studies of SAC related proteins, the question how molecular events at kinetochores become integrated to inhibit or allow segregation of replicated chromosomes has remained one of the big mysteries of the cell cycle [12, 24]. But proteins aggregate, such as in MTs that mediate the position-dependent forces underlying the spatial coherence (alignment) [11], and the clustering of topological links that mediates the compaction force in chromatin [49]. This takes place at different sites in all SC pairs and therefore requires a collective physical treatment. Like all other checkpoints, SAC has to ensure that all upstream phenomena have been completed before the transition to the next phase takes place [1–7, 19]. However, from a physical point of view, this is not a mystery, because such all-or-none type collective phenomena are common in statistical and condensed matter
physicists [63, 64]. Phase transitions then typically depend on temperature, in which case the systems are so-called ‘thermotropic’ [65]. By contrast, cell cycle progression is driven by the interactions of MTs with kinetochores [6], and of chromatin with cohesin, condensins, histones [29, 30, 32, 46] and many other factors. Such systems, driven by various (reactant) concentrations, are called ‘lyotropic’ [65].

Theoretical physicists are normally not interested in such ‘too complex’ biological systems, and neither are molecular biologists in the theory of collective phenomena. Apart from this disciplinary gap, there is also a fundamental statistical physics shortcoming that has prevented physicists from addressing the SAC problem. The dynamics that holds bioriented SC pairs together inhibits segregation only as long as the number of stably attached kinetochore complexes increases. But physical science has only been able to deal with systems containing a constant number of particles (molecular complexes) that are strongly correlated in space [63, 64], or either a fluctuating number of such particles (fugacity) [63], or systems void of strong spatial correlations [66]. Equation (6) of the actual system corresponds formally to the grand partition function of a Bose–Einstein gas locked in a certain kinetic energy state [63] and \( \eta \)a is related to the fugacity. However, in this case \( \eta \)a is regulated by the non-equilibrium reaction (equation (2)). The solution to this problem, was also used in the previous version of the model [25].

Kinetochores were then treated as rigid point-like complexes of proteins, and the higher order folding of chromatin by condensins was taken for granted. Nevertheless, already that simple model provided a suitable many-body physics platform to describe the essential collective properties of the system. Stable and unstable attachments could be separated from each other after a topological quantization [25, 49, 51, 54]. Also in this non-equilibrium lyotropic model, the collective potential energy \( V(\xi) \) (see the appendix) is derived from the three first principles, equations (1), (2a), (2b) and (6), the integrated force \( F_{\text{MT}}(\xi) \) from the gradient of \( V(\xi) \) (like in equilibrium systems), and the oscillatory spectrum from the second derivative of \( V(\xi) \) [54]. The relationship in equation (17), between equation (14) and single molecule force-extension data [45], showed that the integrated force obtained from the collective model is not of the Hooke or WLC type [42, 61], except for small stretches (\( \xi << 1 \)) of chromatin [69, 70]. But since the radius of gyration [41, 43] is valid for arbitrary stretches in the spindle, equations (15), (19) and (24) should provide a good approximation of the integrated (collective) forces and tension at work in a living cell [24]. Knowledge of the \textit{in vivo} tension is in turn key to understanding how a cell decides to collectively inhibit or allow the ‘all-or-none’ anaphase onset. Note that collective phenomena are effects beyond individual molecular properties (a well-known physical fact). Yet, such properties can modify the collective properties, for instance the elasticity, and the conductivity of electricity and mechanical waves (sound), to mention just a couple.

To study the intra-kinetochore stretch and tension, in this generalized collective model kinetochore complexes were divided into two protein complexes, and the integrated MT mediated force \( F_{\text{MT}}(\xi) \) was divided into one integrated poleward force \( F_{\text{p}}(\xi, \xi) \) and one integrated antipoleward force \( F_{\text{a}}(\xi, \xi) \) (figure 4). From these two opposing forces, the integrated MT mediated tensions in kinetochores (equation (20)) and centromeric and pericentromeric chromatin (equation (21)) were obtained, as functions of \( \xi \) and \( q \). It turned out that these two tensions derived from the model were unequal and different from \( F_{\text{p}}(\xi, \xi) \). But this could be expected because the compaction force, which was found to be formally equal to \( F_{\text{MT}}(\xi) \), was not included. The requirement \( F_{\text{p}}(\xi, \xi) = F_{\text{a}}(\xi, \xi) \) is the strengths of \( F_{\text{MT}}(\xi) \) and the compaction force should be equal, hence \( F_{\text{MT}}(\xi)_{\text{max}} = 0.4 \) pN, yielded that \( q = 10 \) and \( k_0 = 2.2 \) pN (see the appendix) and that \( F_{\text{coh}}(\xi) = -F_{\text{MT}}(\xi) \). By addition of \( F_{\text{coh}}(\xi) \) to \( F_{\text{p}}(10, \xi) \), the tensions in kinetochores and chromatin become equal to \( F_{\text{p}}(10, \xi) \) in all SC pairs (equation (24)), as required (all forces should balance each other). This shows that the model is self-consistent, and that the dynamics of the stably connected part of the system, except for short transient periods of turmoil after attachments/detachments of MTs, cohesin, condensin I and histones, actually is spatially coherent. In principle, \( F_{\text{p}}(\xi, \xi) \) and \( F_{\text{a}}(\xi, \xi) \) could also contain a constant term and higher order symmetric terms, which would contribute to tension but not to \( F_{\text{MT}}(\xi) \) because of the opposite signs of such terms. However, since \( F_{\text{p}}(\xi, \xi) \) also equals the tension in chromatin, the constant and higher order terms can be excluded, like in the force-extension curve [45].

Apart from providing the ‘realistic’ parameter values, \( q = 10 \) and \( k_0 = 2.2 \) pN, the separate treatments of the integrated MT and condensin I mediated forces can also be employed to elucidate dysfunctions in segregation processes that may lead to cancer [71–75]. It is known that condensin I depletion can cause double strand breaks in anaphase [71, 72]. In such cases there is no compaction force to be added in equations (22) and (23), implying that the derived tension in kinetochores could, at least for a short time, differ from that in chromatin and also from the integrated poleward forces \( F_{\text{p}}(10, \xi) \). But then the cohesin–chromatin interaction \( F_{\text{coh}}(\xi) \), which should have the same form and strength as \( F_{\text{MT}}(\xi) \), becomes added to \( F_{\text{p}}(10, \xi) \) in equations (22) and (23), making tension in kinetochores and chromatin equal to \( F_{\text{p}}(10, \xi) \) also in that case. However, in prometaphase and metaphase, \( F_{\text{coh}}(\xi) \) contributes only via the second term in equation (1), by disruption of cohesin caused by the increased stretching of chromatin. This implies that the dynamical system can become unstable and anaphase delayed [27, 53]. SC pairs are then unable to align [74] and undergo abnormal movements [73]. Moreover, in the absence of condensin I, since the weakened chromatin then has to be more stretched to reach the same level of tension, the spindle becomes slightly longer [53]. However, \( F_{\text{coh}}(\xi) \) contributes also when condensin I is present; it then works in an alternating synergistic manner together with \( F_{\text{coh}}(\xi) \) [32]. In these derivations the turnover of histones, which should generate a force \( F_{\text{hist}}(\xi) \) of the same form as \( F_{\text{coh}}(\xi) \) too, has been treated as enslaved. But on a shorter time scale, in order to make tension equal in all parts, it may be speculated that \( F_{\text{hist}}(\xi) \) could work in a similar alternating manner together with \( F_{\text{coh}}(\xi) \) and \( F_{\text{coh}}(\xi) \).

As centromeres become extremely stretched [72] and SC pairs fail to align [74] in condensin I depleted cells, the radius of gyration and stretch factor contributes also in higher eukaryotic cells. The extreme stretching in chromatin can also be
expected to pull bioriented SC pairs closer to the polar axis (figure 1(b)). Hence, with \( \theta = 0.75 \) in \( G(\xi, \theta) \), the tension could then in principle reach the critical (abnormal) level of 10 pN (figure 4(d)) where chromatin becomes disrupted [31]. Another risk with overstretched chromatin [72] and longer spindle [53] is that \( \Delta \equiv L/N \) becomes increased, implying that the energy threshold to enter anaphase becomes correspondingly lowered (as explained here above). The system could then become critically sensitive to tensions even much below 5 pN. A third risk that may contribute to abnormal movements of stably attached kinetochores [73] is that the increase in \( \Delta \) can distort the oscillatory spectrum by lowering the frequencies too much. Taken together, these disturbances could be expected to put the genome integrity at stake [72].

The spatial coherence of equation (6), obtained by contracting all \( x_i \) to \( x_{cm} \) in equation (5), implies that the collective SAC dynamics, including interactions of chromatin with cohesin [29, 30], condensin I [49–52] and securin [12, 18, 23, 34–36], becomes spatially coherent too. Given that the compaction of chromatin by condensin I can be described by topological links between different parts of chromatin [49], the maximal compaction at the end of metaphase becomes defined by the topological quantum number \( 2a(\alpha)N \), where \( \alpha \) is the number of bound condensin I molecules per chromosome. This degree of stiffness is apparently needed to induce the final increase in intra-kinetochore distance and the swift anaphase onset [26, 27, 53, 72]. When condensin I is depleted, or cells are treated with low doses of nocodazole, the chromosome integrity can be compromised [71, 72] by the suppressed intra-kinetochore stretch, the increased inter-kinetochore distance and the delayed anaphase onset [27, 53].

But why cannot \( F_{conf}(\xi) \) alone establish normal tension in chromatin? First, recall that the collective compaction dynamics depends on the spatial correlations between stably attached kinetochores and is hence not independent of the MT mediated dynamics. Moreover, chromatin becomes stretched by the two (opposing) integrated MT mediated poleward forces \( F_p(10, \xi) \), which has to be added to the ‘pure’ collective compaction force in chromatin. Precisely this has been done in reversed order in equations (22) and (23). A similar scenario takes place when condensin I is depleted, except that the stretching force then becomes balanced directly by the cohesion between SCs. Tension then depends on the stiffness of scaffolding created closer to the chromatid axis by condensing II in prometaphase [76–80]. Needless to say, all numbers employed here must be treated with caution. It is noteworthy to state that a change in one parameter in the collective model inevitably causes changes in the other parameters. For instance, the value of \( q \) will change with the value of \( F_p(10, \xi)_{\text{max}} \). In this way, the model can exclude data that are not compatible with the prevailing dynamical constraints (first principles), the amounts of available MT plus-ends and vacant kinetochores, the actual time evolution of the system regulated by the attachment rate equation that allows the separation of stable and unstable attachments, and the strong spatial correlations between stably attached kinetochores.

It may seem odd to treat non-locally acting polar ejection forces together with unstable MT attachments. However, the contraction of all sites \( x_i \) to \( x_{cm} \) in equation (6), which is corrected for by the form-factor \( G(\xi, \theta)^2 \), implies that all MT plus-ends become ‘directly’ attached to the kinetochores. Therefore, this non-local part of the SAC dynamics acts like a local interaction. This type of approximation holds in sufficiently rigid condensed matter systems, provided that the time scale is not too short. The contraction point \( x_{cm} \) can then be thought of as a ‘key-hole’ and the \( G(\xi, \theta)^2 \) form-factor as a ‘lens’ through which collective phenomena can be studied and described in terms of properties and functions of individual molecules.

In metaphase and anaphase, when tension approaches zero, the inhibition mechanism becomes more sensitive to disturbances. The equalization of tension implied by the collective potential energy \( V(\xi) \) may then not be sufficient to inhibit anaphase onset. To understand the critical role the time-dependent part of the collective dynamics plays in the SAC machinery, note that without spatial correlations the bioriented SC pairs would oscillate independently. However, with strong spatial correlations, these oscillations become synchronized via the second derivative of \( V(\xi) \). Thus the SAC mechanism becomes more precise with oscillations than without. Given the strictly synchronized oscillations, the collective increase in intra-kinetochore distance at the end of metaphase automatically synchronizes the transition into anaphase [26, 27] by simultaneously changing the metaphase oscillation and branch-point into the corresponding states in anaphase. With anaphase oscillations at \( f_{1A} = (1.08 \pm 0.44) \text{min}^{-1} \) strictly separated from the anaphase branch-point ‘frequency’ at \( f_{\text{bp}} = (1.24 \pm 0.51) \text{min}^{-1} \), there should be no risk of mistakes. Hence the decision to enter anaphase is reduced to a question of energy, as to whether such a transition is energetically possible or not.

The generalized collective interaction thus provides a model for how the SAC mechanism can inhibit or allow anaphase onset both in normal and abnormal cases. In addition to the collective interactions of kinetochores with MT plus-ends, of chromatin with cohesin, condensin I and histones, and of separate with cohesin, the putative in vivo tension \( T(\xi) \) in equation (24), and the relationship in equation (17) to single-DNA molecule force-extension data [45], the generalized collective model also provides a solution to the long-elusive tension-attachment controversy [7]. As is clear from equation (18), it also yields a non-linear extension of the linear elasticity regime of the effective spring constant \( k_0 = 4\varepsilon A g^2 \), i.e. of Young’s modulus [53, 69]. However, the links between the increase in intra-kinetochore stretch and the release of Cdc20 from Mad and Bub proteins in MCC [18, 34, 35, 81], between Aurora B and chromosome condensation induced by condensin I [82, 83], charge dependence of forces that drives DNA condensation [84], and the dependence of forces and tension(s) on the dynamic instability of MTs [81, 85, 86], are but some questions that remain to be further studied at molecular level. The final separation of SCs could be regarded as a well ordered ‘melting’ of SC pairs, mediated by the metaphase and anaphase oscillations and the increase in intra-kinetochore stretch. This is not unlike what happens in thermotropic systems (compare the Debye–Waller factor and Lindemann’s melting formula) [64]. However, oscillations and lattice displacements are then driven by temperature.
Another important SAC function is to repair damaged DNA before anaphase entry, by de novo formation of cohesion between SC pairs and (re-)replication of DNA [87–90]. However, also the non-equilibrium collective interaction, which triggers S phase entry and DNA replication [68], is formally equal to the collective spindle–chromosome interaction in equation (15) which controls the SAC machinery. The dynamics triggering the passage of the G1 restriction point, i.e. the irrevocable commitment to replication [91], is formally equal to the collective SAC dynamics too [67]. In principle, the collective SAC dynamics can thus replicate damaged DNA without essentially changing the collective dynamics, which may also provide a realistic physical picture of the transfer and equalization of energy via MTs [86]. However, in replication the key carriers of interaction are the origin recognition complex (Orc) and the origin of replication [68, 90]. Although a lot more remains to be done, this generalized collective model interaction could hopefully lead to a better understanding of both the normal and abnormal division of cells [12, 24, 72]. It might also lead to a better understanding of the interaction of chromatin with epigenetic and cancerous factors and thereby to development of better anti-cancer drugs.

Acknowledgments

I thank Anders Hambberger, Christer Höög, Anna Kouznetsova, Danko Radic and Tala Stanne for valuable discussions and a critical reading of the manuscript.

Appendix.

A.1. Dynamic reactant concentrations

The variables \( \rho_K \equiv a(1 + \frac{1}{K}) \geq \rho_0 \) and \( \sigma_K \equiv a(1 - \frac{1}{K}) \leq \sigma_0 \), where \( a = (\rho_0 + \sigma_0 + K)/2 \), \( b^2 = (a^2 - b^2)/\sigma_0^2 \), and \( K = k'/K \), can first be regarded as labour-saving shorthand notations when the initial boundary conditions in equations (2a) and (2b) are inserted in equation (1), and the solution in equation (4) is then obtained from equations (3a) and (3b). The advantage becomes even more obvious when equations (3a) and (3b) are combined with the strong spatial correlations defined by equation (6). To understand that \( \rho_K, \sigma_K, \rho' \) and \( \sigma' \) actually represent the dynamic reactant concentrations, it is instructive to verify that they become the usual ‘bare’ concentrations \( \rho_0 \), \( \sigma_0 \), \( \rho \), \( \sigma \) in the high affinity limit, \( k' = 0 \). The dynamic concentrations \( \rho'(x, t) = \rho_K - \psi(x, t) \) and \( \sigma'(x, t) = \sigma_K - \psi(x, t) \) then yield the usual initial boundary constraints defined by equations (2a) and (2b), \( \rho(x, t) = \rho_0 - \psi(x, t) \) and \( \sigma(x, t) = \sigma_0 - \psi(x, t) \).

A.2. Hooke’s law and harmonic oscillations

A small mass \( m \) attached to a spring obeys Hooke’s law, \( F(x) = -k_{\text{H}}x \), where \( F(x) \) is the applied (linear) stretching force, \( k_{\text{H}} \) the ‘Hookean’ spring constant and \( x \) the stretch of the spring. This is the so-called harmonic oscillator and its equation of motion

\[
m \frac{d^2 x}{dt^2} = -k_{\text{H}} x, \quad (A1)
\]

has solutions of the type \( x = x_0 \sin(2\pi f t) \), or \( x = x_0 \cos(2\pi f t) \), where the frequency \( f \) is given by \( (2\pi f)^2 = k_{\text{H}}/m \). However, as described in this report, the oscillatory spectrum of the non-linear SAC dynamics is determined by the second derivative of the collective potential.

Apart from the discrete oscillation mode, the system of SC pairs, stably connected by MTs to the two spindle poles, also contains a so-called branch-point. This can be interpreted as a discrete ‘would-be’ oscillatory state, in which the binding between the sisters has become too weak, apparently because of too much loss of cohesion, to remain bound. The kinetic energy then exceeds the binding energy and the system of SC pairs therefore becomes segregated.

A.3. The displacive force

In a string, to which several masses are attached with spacing \( \Delta x \), the mass at site \( x \) cannot be moved without moving also the neighbouring masses at \( x + \Delta x \) and \( x - \Delta x \), creating a so-called displacive force, which is described below. In principle, the stably attached kinetochore complexes can be spread arbitrarily in the spindle volume of length \( L \) and cross-section area \( A \), but in the continuum approximation [46] the spacing between the kinetochore complexes is set equal to zero. The time-dependent part of equation (14) then equals that of an elastically braced string [47] or a rigid rod in which elastic waves propagate.

To describe the displacive force in a rigid rod, where the mass is distributed continuously in space, a small volume element between \( x \) and \( x + \Delta x \) is extracted (figure A1(a)). When the rod is stretched by an applied (external) force, the volume element becomes deformed and displaced as in figure A1(b) between the two cross-section areas at \( x + \xi \) and \( x + \Delta x + \xi' \). Assume that the applied force has the strengths \( F' \) at \( x + \xi \) and \( F'' \) at \( x + \Delta x + \xi' \).

According to Hooke’s law, the average force \( F = (F' + F'')/2 \) then becomes
\[
\frac{F}{A} = \frac{\xi' - \xi}{\Delta x} \approx \frac{d\xi}{dx}, \quad (A2)
\]
where \( \epsilon \) is the elastic (Young’s) modulus. Newton’s second law yields

\[
\Delta F = \frac{F'' - F'}{\Delta x} = \rho A \Delta \nu \frac{d^2 \xi}{dx^2}. \quad (A3)
\]
where \( \rho \) is the mass density of the rod.

After division by \( \Delta x \), in the limit \( \Delta x \to 0 \), equation (A3) becomes

\[
\frac{dF}{dx} = \rho A \frac{d^2 \xi}{dx^2}. \quad (A4)
\]
The spatial derivative of equation (A2)

\[
\frac{dF}{dx} = \epsilon A \frac{d^2 \xi}{dx^2}, \quad (A5)
\]
inserted in equation (A4) then yields

\[
\frac{dF}{dx} = \frac{\rho}{\epsilon} \frac{d^2 \xi}{dx^2}. \quad (A6)
\]
This explains the presence of the second-order spatial derivative, the so-called displacive force term, in equation (14), where \( \eta = A \rho \) is the linear mass density of the rod, \( e = \epsilon \), and is \( \nu + \) the velocity for the propagation of lattice oscillations.

The time-independent part of equation (14) (multiplicative constants are dropped),

\[
\frac{d^2 \xi}{dx^2} = -\frac{\nu}{\epsilon}, \quad (A7)
\]
multiplied by \( d\xi/dx \) gives

\[
\frac{d\xi}{dx} \frac{d^2 \xi}{dx^2} = \frac{d\nu}{d\xi} \frac{d^2 \xi}{dx^2} = \frac{d\nu}{d\xi}, \quad (A8)
\]
which yields in turn

\[
\frac{d}{dx} \left( \frac{d\xi}{dx} \right)^2 = \frac{d(2\nu)}{d\xi} \frac{d\xi}{dx} = \frac{d(2\nu)}{dx}. \quad (A9)
\]
This shows that

\[
\left( \frac{d\xi}{dx} \right)^2 = 2\nu, \quad (A10)
\]
hence

\[
\frac{d\xi}{dx} = \sqrt{2\nu}, \quad (A11)
\]
which was used to obtain the (lyotropic) collective potential energy from equation (10).

### A.4. Decomposition of the integrated force

The integrated MT mediated force \( F_{\text{MT}}(\xi) \) in equation (14) is skew-symmetric about \( \xi = 1 \), suggesting that the underlying integrated MT mediated poleward and antipoleward forces are skew-symmetric too. With \( \kappa = 1 \), a third-order polynomial representing the skew-symmetric antipoleward force \( F_a(q, \xi) \), which vanishes at \( \xi = 0 \) and 2, can be written as

\[
F_a(q, \xi) = \frac{a_c}{2} (\xi - 2)(\xi - q), \quad (A12)
\]
where \( q > 2 \). The assumption that \( F_a(q, \xi) \) has a central symmetry with respect to \( \xi = 1 \) and \( F_a(q, \xi) \) implies that

\[
F_a(q, \xi) = -F_a(q, 2 - \xi), \quad (A13)
\]
which yields

\[
F_a(q, \xi) = \frac{a_c}{2} (\xi - 2)(\xi - q), \quad (A14)
\]
The constant \( a_c \) is determined by the restriction that the integrated MT mediated antipoleward and poleward forces added together must yield the skew-symmetric integrated force \( F_{\text{MT}}(\xi) \) defined by equation (14), hence

\[
F_{\text{MT}}(\xi) = F_a(q, \xi) + F_a(q, 2 - \xi), \quad (A15)
\]
from which follows that \( a_c = -1/2 \) \( (q > 2) \). Accordingly,

\[
F_a(q, \xi) = -\frac{1}{2} \frac{a_c}{2} (\xi - 2)(\xi - q), \quad (A16)
\]
which inserted in equation (A15) yields

\[
F_{\text{MT}}(\xi) = -\frac{1}{4} \frac{a_c}{2} (\xi - 1)(\xi - 2). \quad (A18)
\]

### A.5. Energy threshold blocking anaphase entry

At the end of prometaphase, where \( \xi = 2 \) in the continuum model [46], it is assumed that the turnover from all MT attachments has relaxed except for the last two attachments in the last bioriented SC pair in budding yeast (\( \xi_{\text{th}} = 2 - 2/16 = 1.875 \)), and except for the last six in the three last bioriented SC pairs in higher eukaryotes (\( \xi_{\text{th}} = 2 - 6/46 = 1.870 \)). For simplicity, the value \( \xi_{\text{th}} = 1.875 \) is used in all types of cells. The energy \( \Delta E_{\text{MA}} \) required to enter anaphase from metaphase, by which the intra-kinetochore stretch \( \delta \) decreases from \( \delta_{\text{th}} = 65 \text{ nm} \) to \( \delta_{\text{th}} = 100 \text{ nm} [26] \), can be calculated from the potential energy \( V(\xi_{\text{th}}) = D'\kappa_{\text{MT}}(2\xi_{\text{th}}^2 - D/\delta_{\text{th}}^2)/8 \), where \( C_{\text{th}} = \xi_{\text{th}}^2(2 - \xi_{\text{th}}^2) \) and \( \Delta = \delta. \) To increase the intra-kinetochore stretch from \( \delta_{\text{th}} \) to \( \delta_{\text{th}} \) in anaphase, the system thus spends a potential energy \( \Delta V_{\text{MA}} = C_{\text{th}} D'\kappa_{\text{MT}}(2\delta_{\text{th}}^2 - D/\delta_{\text{th}}^2)/8 \) in metaphase. But \( \Delta V_{\text{MA}} \) should also equal the decrease in energy gap between the oscillatory state and the branch-point, this energy gap being \( E_0 = C_{\text{th}} D'\kappa_{\text{MT}}(2\delta_{\text{th}}^2 - f_1^2) = C_{\text{th}} D'\kappa_{\text{MT}}(2\delta_{\text{th}}^2 - f_1^2)/2 = C_{\text{th}} D'\kappa_{\text{MT}}(2\delta_{\text{th}}^2 - f_1^2)/2 = C_{\text{th}} D'\kappa_{\text{MT}}(2\delta_{\text{th}}^2 - f_1^2)/2 = C_{\text{th}} D'\kappa_{\text{MT}}(2\delta_{\text{th}}^2 - f_1^2)/2 \).

\[
E_0 = C_{\text{th}} D'\kappa_{\text{MT}}(2\delta_{\text{th}}^2 - f_1^2)/2. \quad (A19)
\]
Table A1. Columns 1, 2, and 3 show how the peak of the integrated MT mediated antipoleward force changes position (column 2) and strength (column 3) for different values of $q$ (column 1). In columns 4 and 5, the effective spring constant $k_0$ and the amplitude of the integrated MT mediated force $F_{\text{MTmax}}$ are calculated for the same values of $q$, when $F_{\text{pmax}} = -F_{\text{amin}}$ is kept fixed at 7.704 pN. When $F_{\text{pmax}} = -F_{\text{amin}}$ are instead fixed at 5.0 pN, the values in column 4 and 5 decrease correspondingly by a factor 5/7.704 = 0.649. When $q = 10$, $k_0$ decreases from 3.4 to 2.2 pN and $F_{\text{max}}$ from 0.657 to 0.4 pN, which thus equals the amplitude of the compaction force.

| $Q$ | $\xi$ | $F_{\text{amin}}$ (pN) when $k_0 = 1$ | $k_0(q)$ (pN) | $F_{\text{MTmax}}$ (pN) |
|-----|------|---------------------------------|--------------|-------------------|
| 2.00 | 0.667 | -0.297 | 25.981 | 5.000 |
| 2.01 | 0.668 | -0.299 | 25.807 | 4.966 |
| 2.1  | 0.683 | -0.319 | 24.176 | 4.653 |
| 2.5  | 0.736 | -0.410 | 18.778 | 3.614 |
| 3.0  | 0.785 | -0.528 | 14.586 | 2.807 |
| 5.0  | 0.880 | -1.015 | 7.589 | 1.460 |
| 6.0  | 0.903 | 1.262 | 6.103 | 1.175 |
| 7.0  | 0.918 | -1.510 | 5.101 | 0.982 |
| 10.0 | 0.945 | -2.257 | 3.413 | 0.657 |

A.6. Parameter values

The question is then how to determine the most realistic values of $q$ and $k = k_0G^2$ in equations (A16) and (A17). For simplicity it is assumed that $G(\xi, 0)^2 = 1$ in these derivations. Column 1 in table A1 starts with $q = 2.001$, because the largest separation in $\xi$ (the most skew-symmetric case) (column 2) between $F_{\text{amin}}$ and $F_{\text{pmax}}$ (figure 4(a)), is obtained when $q$ approaches 2.0 from above. This case is chosen only to illustrate the asymmetry of forces, which becomes less obvious at higher values of $q$. With $k_0 = 1$, the integrated force $F_{\text{MT}}(\xi)$ in equation (14) attains its minimum, $-0.192$ pN at $\xi = 0.423$. To decrease this minimum to $-5$ pN [28], the effective spring constant must be increased from $k_0 = 1$ to $k_0 = 25.981 = 26$ pN (column 4 in table A1). With $k_0 = 1$, the antipoleward force $F_{\text{d}}(q, \xi)$ attains its minimum $-0.297$ pN (column 3) at $\xi = 0.667$ (column 2). When $k_0$ is increased to 25.981 = 26 pN (figure 4(a)), this minimum decreases to $-7.704$ pN. All forces are thus still kept below the 10 pN by which chromatin becomes disrupted [26]. Both the position in $\xi$-space (column 2) and the value of $F_{\text{d}}(\xi)$ (column 3) depend on $q$ (column 1). By adjusting the effective spring constant $k_0(q)$ (column 4) concomitantly with the changes in $q$ (column 1), $F_{\text{amin}} = -F_{\text{amin}}$ can be held fixed at 7.704 pN. $F_{\text{MT}}(\xi)$ then decreases below 5 pN according to column 5. This case is more realistic because it creates less imbalance between the integrated poleward force $F_{\text{d}}(q, \xi)$ and the antipoleward force $F_{\text{d}}(q, \xi)$, and enables metaphase oscillations to start earlier. However, the replicated chromosomes are ultimately separated by the two opposing integrated forces $F_{\text{d}}(q, \xi)$ directed towards the two spindle poles. It therefore seems more realistic to require that $F_{\text{d}}(q, \xi)_{\text{max}} = -F_{\text{d}}(q, \xi)_{\text{min}} = 5$ pN [28] and to let $F_{\text{MT}}(\xi)$ decrease below 1 pN. $F_{\text{d}}(q, \xi)$, $F_{\text{d}}(\xi)$ and $F_{\text{MT}}(\xi)$ then relax more rapidly, and metaphase oscillations can start without delay.

The compaction force $F_{\text{comp}}(\xi)$ has been shown to be formally equal to $F_{\text{comp}}(\xi)$. Together with the requirement $F_{\text{MT}}(\xi)_{\text{max}} = 0.4$ pN, this implies that $F_{\text{comp}}(\xi) = -F_{\text{MT}}(\xi)$. By addition of $F_{\text{comp}}(\xi)$ to the integrated antipoleward force $F_{\text{d}}(q, \xi)$ in equations (22) and (23), the tension in kinetochores then becomes equal to the tension in chromatid and to $F_{\text{p}}(q, \xi)$, showing that $F_{\text{MT}}(\xi)_{\text{max}} = 0.4$ pN is an adequate strength of $F_{\text{MT}}(\xi)$ and that the derivation as such is self-consistent. This is because tension should be equal from pole to pole. This could have been said already from the start, but the separate treatments of MT and condensin I mediated forces yielded that $q = 10, k_0(10) = 2.2$ pN and thus also determined the strength of $F_{\text{p}}(10, \xi)$. It also showed that $F_{\text{comp}}(\xi)$ plays a significant role in the SAC dynamics, in particular in metaphase where it contributes to the increase in intra-kinetochore distance. Moreover, it may help to reveal what can happen in condensin I depleted cells. Obviously, when $G(\xi, 0)^2 \neq 1$, all obtained numbers become modified, which is also the case if the force required for chromosome segregation is different from 5 pN.

References

[1] Murray A W and Kirschner M W 1989 Science 246 614
[2] Li R and Murray A W 1991 Cell 66 519
[3] Hartwell L H and Weinert T A 1989 Science 246 629
[4] Murray A W 1992 Nature 359 599
[5] Meraldi P, Draviam V M and Sorger P K 2004 Dev. Cell 7 45
[6] Rieder C L, Schultz A, Cole R and Stu1der G 1994 J. Cell Biol. 127 1301
[7] Pinsky B A and Biggins S 2005 Trends Cell Biol. 15 486
[8] Maresca T J and Salmon E D 2010 J. Cell Sci. 123 825
[9] Li X and Nicklas R B 1995 Nature 373 630
[10] Nicklas R B 1997 Science 275 632
[11] Mitchison T J and Salmon E D 2001 Nature Cell Biol. 3 E17
[12] Peters J-M 2007 Nature 446 868
[13] Kops G J P L, Weaver B A A and Cleveland D W 2005 Nature Rev. Cancer 5 773
[14] Straight A F, Marshall W F, Sedat J W and Murray A W 1997 Science 277 574
[15] Cheeseman I M and Desai A 2008 Nature Rev. Mol. Cell Biol. 9 33
[16] King R W, Peters J-M, Tugendreich S, Rolfe M, Hieter P and Kirschner M W 1995 Cell 81 279
[17] Sudakin V, Ganoth D, Dahan A, Heller H, Hershko J, Luca F C, Ruderman J V and Herskho A 1995 Mol. Cell. Biol. 6 185
[18] Peters J-M 2006 Nature Rev. Mol. Cell Biol. 7 644
[19] Hoyt M A, Totis L and Roberts B T 1991 Cell 66 507
[20] Waters J C, Chen R-H, Murray A W and Salmon E D 1998 J. Cell Biol. 141 1181
[21] Howell B J, Hoffmann D B, Fang G, Murray A W and Salmon E D 2000 J. Cell Biol. 150 1233
[22] Sudakin V, Chen G K T and Yen T J 2001 J. Cell Biol. 154 925
[23] Musacchio A and Salmon E D 2007 Nature Rev. Mol. Cell Biol. 8 379
[24] Lau D T C and Murray A W 2012 Curr. Biol. 22 180
[25] Mattson L 2009 J. Phys.: Condens. Matter 21 502101
[26] Maresca T J and Salmon E D 2009 J. Cell Biol. 184 373
[27] Uchida K S K, Takagaki K, Kumada K, Hirayama Y, Noda T, Ueda H, Hirota T and Hirota T 2009 J. Cell Biol. 184 383
[28] Wan X et al 2009 Cell 137 672
[29] Guacci V 2007 Genes Cells 12 693
[30] Nasmyth K and Haering C H 2009 Annu. Rev. Genet. 43 525
[31] Strick T R, Kawaguchi T and Hirano T 2004 Curr. Biol. 14 874
[32] Losada A and Hirano T 2005 Genes Dev. 19 1269
[33] Verdaasdonk J S and Bloom K 2011 Nature Rev. Mol. Cell Biol. 12 320


[34] Reddy S K, Rape M, Margansky W A and Kirschner M W 2007 Nature 446 926

[35] Stegmeier F et al 2007 Nature 446 876

[36] Uhlmann F, Lottspeich F and Nasmyth K 1999 Nature 400 37

[37] Rieder C L, Cole R W, Khodjakov A and Sluder G 1995 J. Cell Biol. 130 941

[38] Keung S, Hegemann B, Peters B H, Lipp J J, Schleiffer A, Mechtler K and Peters J-M 2006 Cell 127 955

[39] Liu J, Desai A, Onuchic J N and Hwa T 2007 Proc. Natl Acad. Sci. USA 104 16104

[40] Maiato H, DeLuca J, Salmon E D and Earnshaw W C 2004 J. Cell Biol. 167 5461

[41] Flory P J 1989 Statistical Mechanics of Chain Molecules (Munich: Hanser)

[42] Doi M and Edwards S F 1986 The Theory of Polymer Dynamics (Oxford: Clarendon)

[43] Debye P 1946 J. Chem. Phys. 14 636

[44] Zhuang X 2004 Science 305 188

[45] Bustamante C, Marko J F, Siggia E D and Smith S 1994 Science 265 1599

[46] Bouck D C and Bloom K 2007 Curr. Biol. 17 741

[47] Verdaasdonk J S, Gardner R, Stephens A D, Yah E and Bloom K 2012 Mol. Biol. Cell 23 2560

[48] Kimura K and Hirano T 1997 Cell 90 625

[49] Cuylen S, Metz J and Haering C H 2011 Nature Struct. Mol. Biol. 18 894

[50] Wood A J, Severson A F and Meyer B J 2010 Nature Rev. Genet. 11 391

[51] Hirano T 2012 Genes Dev. 26 1659

[52] Hudson D F, Marshall K M and Earnshaw W C 2009 Chromosome Res. 17 131

[53] Ribeiro S A et al 2009 Mol. Biol. Cell 20 2371

[54] Jackiw R 1977 Rev. Mod. Phys. 49 681

[55] Combs J A and Yip S 1983 Phys. Rev. B 28 1873

[56] Morse P M and Feshbach H 1953 Methods of Theoretical Physics (New York: McGraw-Hill)

[57] Dumont S, Salmon E D and Mitchison T J 2012 Science 337 355

[58] Pearson C G, Maddox P S, Salmon E D and Bloom K 2001 J. Cell Biol. 152 1255

[59] Matos I, Pereira A J, Lince-Faria M, Cameron L A, Salmon E D and Maiato H 2009 J. Cell Biol. 186 11

[60] Goshima G and Scholey J M 2010 Annu. Rev. Cell Dev. Biol. 26 21

[61] Marko J F and Siggia E D 1995 Macromolecules 28 8759

[62] Matsson L (ed) 1998 Proc. Adriatico Research Conf.: Nonlinear Cooperative Phenomena in Biological Systems (Trieste, 1997) (Singapore: World Scientific)

[63] Reichl L E 1998 A Modern Course in Statistical Physics (New York: Wiley)

[64] Ziman J M 1965 Principles of the Theory of Solids (Cambridge: Cambridge University Press)

[65] De Gennes P G and Prost J 1993 The Physics of Liquid Crystals (Oxford: Clarendon)

[66] Nicolis G and Prigogine I 1977 Self-Organization in Nonequilibrium Systems (New York: Wiley)

[67] Matsson L 1993 Phys. Rev. E 48 2217

[68] Matsson L 2005 J. Biol. Phys. 31 303

[69] Bloom K and Joglekar A 2010 Nature 463 446

[70] Stephens A D, Haase J, Vicci L, Taylor R M II and Bloom K 2011 J. Cell Biol. 193 1167

[71] Samoshkin A, Arnaoutov A, Jansen L E T, Ouspenski I, Dye L, Karpova T, McNelly J, Dasso M, Cleveland D W and Strunnikov A 2009 PLoS ONE 4 e6831

[72] Samoshkin A, Dulev S, Loukinov D, Rosenfeld J A and Strunnikov A V 2012 Chromosoma 121 191

[73] Gerlich D, Hirota T, Koch B, Peters J-M and Ellenberg J 2006 Curr. Biol. 16 333

[74] Oliveira R A, Coelho P A and Sunkel C E 2005 Mol. Cell. Biol. 25 8971

[75] Strunnikov A V 2010 Cell Div. 5 15

[76] Kleckner N, Zickler D and Witz G 2013 Science 342 940

[77] Naumova N, Imakaev M, Fudenberg G, Zhan Y, Lajoie B R, Mirny L A and Dekker J 2013 Science 342 948

[78] Marco J F 2008 Chromosome Res. 16 469

[79] Maeshima K and Eltsov M 2008 J. Biochem. 143 145

[80] Maeshima K and Laemmli U K 2003 Dev. Cell 4 467

[81] Foley AE and Kapoor T M 2013 Nature Rev. Mol. Cell Biol. 14 25

[82] Lipp J J, Hirota T, Poser I and Peters J-M 2007 J. Cell Sci. 120 1245

[83] Tada K, Susumu H, Sakuno T and Watanabe Y 2011 Nature 474 477

[84] DeRouchey J, Parsegian V A and Rau D C 2010 Biophys. J. 99 2608

[85] Pavin N, Laan L, Dogterom M and Jülicher F 2008 Proc. Natl Acad. Sci USA 105 8920

[86] Sataric M V, Tuszynski and Zakula R B 1993 Phys. Rev. E 48 589

[87] Watrin E and Peters J-M 2007 Science 317 209

[88] Ström L, Karlsson C, Lindroos H B, Wedahl S, Katou Y, Shirahige K and Sjögren C 2007 Science 317 242

[89] Ünal E, Heidinger-Pauli J M and Koshland D 2008 Science 321 152

[90] Matsson L 2005 J. Cell Sci. 217 155102

[91] Smith KA 2005 Mol. Biol. Cell 16 955

[92] Maeshima K and Eltsov M 2008 J. Biochem. 143 145

[93] Maeshima K and Laemmli U K 2003 Dev. Cell 4 467

[94] Foley AE and Kapoor T M 2013 Nature Rev. Mol. Cell Biol. 14 25

[95] Pavin N, Laan L, Dogterom M and Jülicher F 2008 Proc. Natl Acad. Sci USA 105 8920

[96] Sataric M V, Tuszynski and Zakula R B 1993 Phys. Rev. E 48 589

[97] Watrin E and Peters J-M 2007 Science 317 209

[98] Ström L, Karlsson C, Lindroos H B, Wedahl S, Katou Y, Shirahige K and Sjögren C 2007 Science 317 242

[99] Ünal E, Heidinger-Pauli J M and Koshland D 2008 Science 321 152