Forces on Chromosomal DNA during Anaphase

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ABSTRACT In the course of anaphase, the chromosomal DNA is submitted to the traction of the spindle. Several physical problems are associated with this action. In particular, the sister chromatids are generally topologically intertwined at the onset of anaphase, and the removal of the intertwinings results from a coupling between the enzymatic action of type II DNA topoisomerases and the force exerted by the spindle. We propose a physical analysis of some of these problems: 1) We compare the maximum force the spindle can produce with the force required to break a DNA molecule, and define the conditions compatible with biological safety during anaphase. 2) We show that the behavior of the sister chromatids in the absence of type II DNA topoisomerases can be described by two distinct models: a chain pullout model accounts for the experimental observations made in the budding yeast, and a model of the mechanical rupture of rubbers accounts for the nondisjunction in standard cases. 3) Using the fluctuation-dissipation theorem, we introduce an effective protein friction associated with the strand-passing activity of type II DNA topoisomerases. We show that this friction can be used to describe the situation in which one chromosome passes entirely through another one. Possible experiments that could test these theoretical analyses are discussed.

INTRODUCTION

Chromosome segregation during anaphase has been studied in great detail in a number of eukaryotic organisms (Alberts et al., 1994). A general problem raised by these studies is that of relating the behavior of the chromosomes to the action of the mitotic spindle. An excellent introduction to this complex problem is provided by the movies illustrating various types of behaviors in living *Haemanthus* (lily) cells (Bajer and Molè-Bajer, 1962). In a normal anaphase (Fig. 1 *A*), the metaphase chromosome undergoes a longitudinal fission, which generates the two daughter chromosomes. The fission process defines anaphase onset. After the fission, the daughter chromosomes pulled apart by the mitotic spindle slowly travel toward the poles.

Irradiation of *Haemanthus* cells produces abnormal chromosomes, the behaviors of which reveal other aspects of anaphase (Fig. 1, *B–D*). Fig. 1 *B* shows a two-kinetochore chromatid resulting from a sister reunion. During anaphase the two kinetochores move toward opposite poles, and the segments between the kinetochores are stretched and finally break, either in anaphase or in telophase. The breakage in anaphase is thought to be due to the pulling action of the spindle, whereas breakage in telophase is due to the division of the cytoplasm by phragmoplast activity (Bajer, 1964). The behavior of dicentric chromosomes is variable. We describe here the cases where the two kinetochores can move to the correct poles, but with the chromatids between them looped around one another (Fig. 1, *C* and *D*); the initial fission generates two daughter chromosomes that are still interlocked. In the case of a single interlocking, one can see "one of the chromosomes passing through the other one, and in spite of this both remaining intact" (Bajer, 1963) (Fig. 1 *C*). If there is more than one interlocking, this passage is not observed and one of the chromosomes breaks (Fig. 1 *D*). Fig. 1 *E* illustrates the segregation of interlocked ring chromosomes in which one can also observe the passage of one chromosome through the other.

These classic cytological observations revealed the existence of various mechanical types of chromosomes in anaphase. A first classification of these different types was in fact proposed long ago by Darlington (1937). From these early microcinematographic studies, it is already clear that to describe the behavior of the chromosomes during anaphase, we must in general consider at least three components: the spindle, the chromosomal thread, and a strand-passing activity. The mechanical properties of the spindle have been studied by Nicklas (Nicklas, 1983; reviewed by Nicklas, 1988), who has determined force-velocity curves and measured the maximum force that the spindle can exert in anaphase. An essential feature of the chromosomal thread or chromonema is its continuity. This is the unineme hypothesis, which states that each chromosome contains a single molecule of double-stranded DNA. The unineme hypothesis means that the phenomenon of chromosome breakage observed in anaphase requires the breakage of a DNA molecule. To analyze the breakage we shall compare the force exerted by the spindle with the tensile strength of DNA (the force necessary to break it) determined by Bensimon et al. (1995). Another consequence of the continuity of the chromosomal thread is that there must exist a strand-passing activity operating by breakage and reunion in the situations described in Fig. 1, *C* and *E*. We now know that this strand-passing activity is provided by type II DNA

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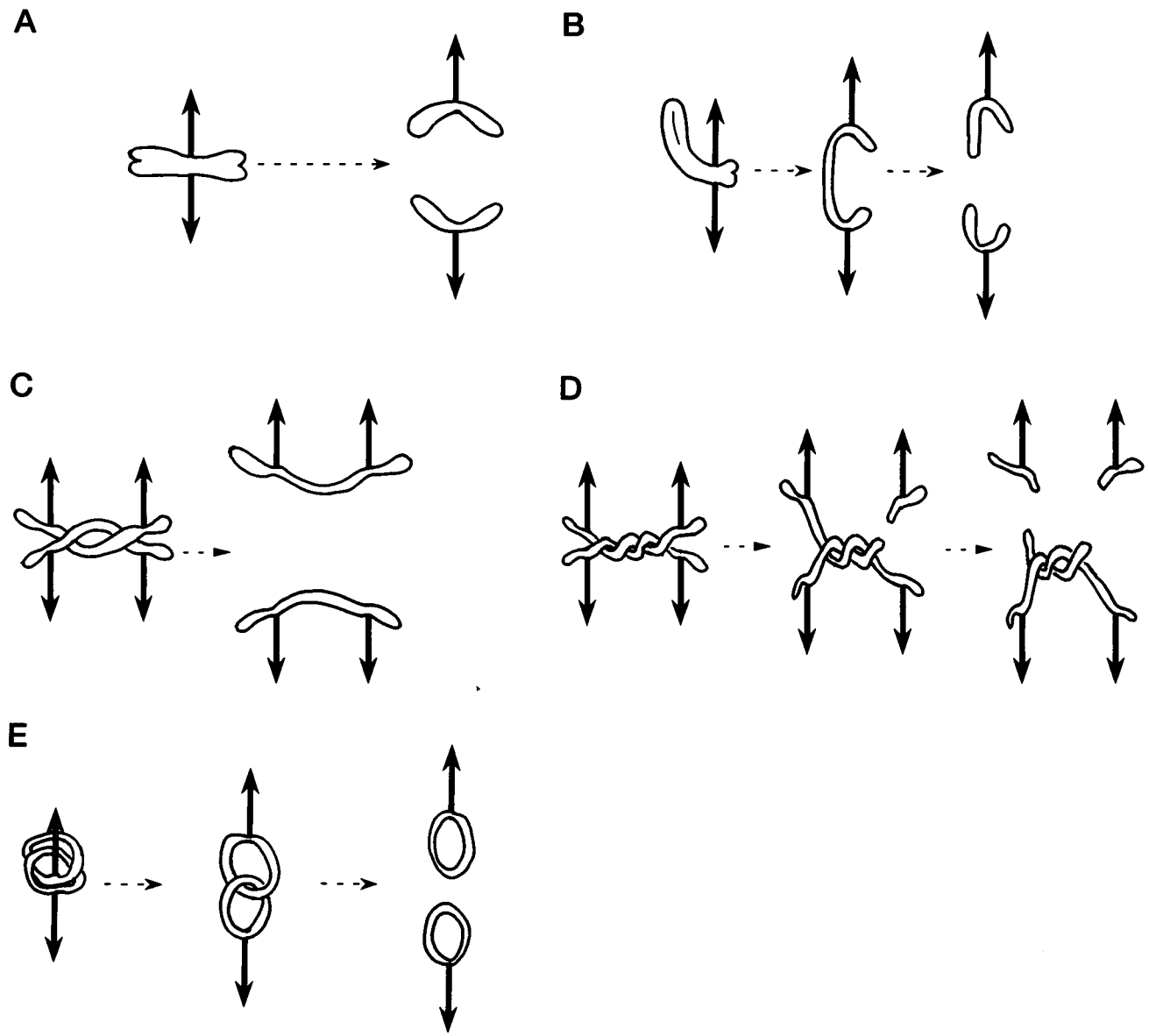

FIGURE 1 Schematic representation of the chromosomal behavior during anaphase in normal (*A*) and irradiated (*B–E*) *Haemanthus* mitotic cells. The chromosomal spindle fibers are represented by thick arrows. Successive events are connected by dotted arrows. The typical size of the chromosomes shown is 10 to 20 μm. (*A*) Normal anaphase. Anaphase onset is defined by the longitudinal fission of the metaphase chromosome. (*B*) Breakage of a sister reunion bridge. The two kinetochores move toward opposite poles. The chromatid bridge between the kinetochores is stretched and finally breaks. (*C* and *D*) Behavior of dicentric chromosomes, in which the two kinetochores move toward the correct poles, but where the regions between the two kinetochores are coiled around one another 360° or more. (*C*) Single interlocking. The two daughter chromosomes are still interlocked after the initial longitudinal fission, and the chromosomes of one pass through the other. Notice that this behavior was erroneously described in our previous work (Duplantier et al., 1995a). It was referred to as a criss-cross configuration, but this is not the case: the criss-cross configuration corresponds to a situation in which the two kinetochores of each daughter chromosome move to the opposite poles, leading to breakage (Bajer, 1963). (*D*) Double interlocking. One of the chromosomes breaks. (*E*) Ring chromosomes. One of the chromosomes passes through the other.

topoisomerase (topo II), an enzyme that catalyzes the passage of double-stranded DNA segments through one another (Liu et al., 1980). That this enzymatic activity could explain the segregation of interlocked ring chromosomes was apparently first noted by Hsieh and Brutlag (1980) and has also been discussed by Murray and Szostak (1985) in a thought-provoking review. Interlocking of chromosomes or bivalents resulting from synapsis or genetic recombination is frequently observed in meiosis (Holm and Rasmussen, 1980; von Wettstein et al., 1984). The role of topo II in the removal of these interlocks has been discussed by Holm and Rasmussen (1980), Rasmussen (1986), and Murray and Szostak (1985).

Recent experiments have shown that topo II is also generally required for the normal mitotic fission process occurring in the linear, monocentric chromosomes described in

Fig. 1 *A* (reviewed by Holm, 1994). In these experiments, when anaphase is initiated in cells lacking topo II activity, fission usually fails, leading to chromosome nondisjunction, chromosome breakage, and cell death. These phenomena have been observed with different approaches in several organisms: with topo II mutants in the budding and fission yeasts (Holm et al., 1985; Uemura and Yanagida, 1986; Uemura et al., 1987; Holm et al., 1989; Funabiki et al., 1993; Spell and Holm, 1994); using topo II inhibitors in mammalian cells (Downes et al., 1991; Clarke et al., 1993; Gorbsky, 1994), *Drosophila* embryos (Buchenau et al., 1993), and in *Xenopus* extracts (Shamu and Murray, 1992); and by microinjection of anti-topo II antibodies into *Drosophila* embryos (Buchenau et al., 1993).

The requirement for topo II is explained by the existence of intertwinings between the two sister chromatids, which result from the replication process. These intertwinings appear to be removed by topo II at the onset of anaphase, when the spindle pulls apart the sister chromatids. Thus there exists a coupling between the enzymatic action of topo II and the traction of the spindle. In a previous work, we investigated the consequences of the coupling on the kinetic of the standard fission process (Duplantier et al., 1995a). The velocity of the chromatids in anaphase must be compatible with the action of topo II: we concluded from our analysis that the highest observed relative velocities (about 0.1 μm s^{-1}; see Mazia, 1961) are close to the theoretical upper bound compatible with the action of the enzyme. In the present work, we extend this analysis to the dynamics of the fission process. We propose models describing chromosomal segregation in the absence and in the presence of topo II. The rheology of a system to which an external force is applied can be described by constitutive equations relating stress and strain. Such relations have proved very useful in modeling the disentanglements of polymeric chains, by using the reptation model (de Gennes, 1979; Doi and Edwards, 1986). In other situations, such as the mechanical rupture of rubber, it is necessary to account for the breakage of chemical bonds. We shall see that chromosomal segregation in the absence of topo II can be handled by either one of these models. In the presence of topo II, a new feature must be included in the model, namely the coupling between chain dynamics and topo II strand-passing activity. We propose below a very crude formulation of this coupling, based on the so-called fluctuation-dissipation theorem (van Kampen, 1992), a result derived from the general principles of equilibrium statistical mechanics. A preliminary account of this work has already appeared (Duplantier et al., 1995b).

FORCES IN ANAPHASE

The force produced by the anaphase spindle has been measured by Nicklas (1983) with a flexible glass needle calibrated in newtons per micron of tip deflection. The needle was used to exert a force on a moving chromosome in anaphase after the fission event. The force opposed that produced by the spindle and allowed the determination of a force-versus-velocity curve. In grasshopper (*Melanoplus sanguinipes*) spermatocytes, on which these experiments were performed, the unperturbed chromosome moves with a constant velocity of about 6 nm s^{-1}. This velocity remains constant until the force opposing movement reaches about 100 pN; above that force the velocity decreases rapidly to zero. The maximum force F_{max} the spindle can produce is approximately 700 pN (±50%); this corresponds to the force required to cease chromosome movement. From these experiments an estimate of the maximum force that can be produced per kinetochore microtubule has been derived. Nicklas estimates that the average number of kinetochore microtubules per chromosome is 15 in this system; the corresponding value for the maximum force per microtubule is thus 47 pN. In the case of the budding yeast, in which there is a single kinetochore microtubule per chromosome (Clarke and Carbon, 1985), this value of about 47 pN should correspond to the maximum force produced by the spindle. Nicklas has also determined the elastic modulus by measuring the force required to stretch the chromosomes. The chromosomes behave as Hookean elastic bodies, then $F = \kappa\Delta\ell/\ell_o$ (Nicklas, 1988), where F is the applied force, κ is the elastic constant (equal to the Young modulus times the cross-sectional area of the chromosome), and $\Delta\ell/\ell_o$, the change in length ($\Delta\ell$) over the original length (ℓ_o), is the strain. The elastic constant κ for one chromatid is 320 pN. The maximum force F_{max} produced by the spindle is therefore not much greater than the force $F' = \kappa$ required to stretch a chromatid twofold.

Chromosome breakage

Let us now compare F_{max} with the force required to break a chromosome. It has long been known that the force exerted by the spindle can exceed the force required to break the chromosomes; this conclusion was reached by Beams and King (1936) in experiments on chick embryo cells and by Bajer in the experiments described above (Bajer, 1963; Bajer and Molè-Bajer, 1972). According to the unineme hypothesis, the breakage of a chromosome requires the breakage of a double-stranded DNA molecule. The force F_{break} required to break a chromosome is therefore expected to exceed the tensile strength of DNA; this force has been determined by Bensimon et al. (1995), who found a value of about 480 pN (±20%). This value is consistent with a lower bound estimate of about 270 pN given by Harrington and Zimm (1965). We shall assume in this work that F_{break} is also equal to 480 pN (which means that the chromosomal DNA chain is not significantly reinforced by the interactions with the chromosomal proteins).

In the cases where chromosome breakage is observed (Fig. 1, *B* and *D*), each chromatid is stretched by two (Fig. 1 *B*) or four (Fig. 1 *D*) chromosomal spindle fibers, and the maximum force they can produce is about 47 × m pN (Fig. 1 *B*) or 47 × 2 m pN, where m is the number of kinetochore microtubules. This force will be sufficient to break a chro-

mosome only if m is greater than about 10 (Fig. 1 *B*) or 5 (Fig. 1 *D*). This is indeed the case for *Haemanthus*, where $m \simeq 70-80$ (Jensen, 1982): the breakage observed can result from the traction of the spindle alone. We expect, in fact, that the maximum force F_{max} in this case is much greater than F_{break}; this is consistent with the observation that breakage can occur without any noticeable effect on the speed of the chromosome. The conclusion than F_{max} exceeds F_{break} also holds true in general for mammalian cells (because m is generally greater than 5; cf. Rieder, 1982).

In contrast, in the case of the budding yeast, the maximum force produced by the spindle is too small to break a DNA chain. Let us consider a situation in this organism where the two sister chromatids (or homologous chromosomes in meiosis) remain linked by one or more DNA chains in anaphase. In that case, the spindle alone will not be able break this bridge; the velocity of the chromatids will drop to zero, and the fission process will abort. This will be the prime result of the traction by the spindle. If DNA breakage is ultimately observed (and this is the case in the experiments of Spell and Holm, 1994), it must involve factors other than the spindle. What can these factors be? As mentioned above, Bajer (1964) has proposed that in plant cells a chromosome can break in telophase under the action of the phragmoplast. In a similar manner, DNA breakage in animal cells could occur during cytokinesis under the mechanical action of the cleavage furrow. Another possibility is that the breakage is catalyzed by an endonuclease that recognizes and cleaves DNA segments rendered accessible (and possibly stretched) by the spindle. These three factors (the spindle, the cleavage furrow, and the endonuclease) could act in conjunction. In this respect, it is interesting to note that in mammalian cells, topo II inhibition not only leads to a failure of chromosomal fission, but also induces apoptosis (programmed cell death) (Walker et al., 1991; Bicknell et al., 1994). A biochemical hallmark of apoptosis is the cleavage of DNA into fragments by endonucleases (reviewed by Bortner et al., 1995). This endonuclease could also be involved in the DNA breakage considered here. The possible relationship between the spindle and apoptosis is discussed in greater detail below.

To sum up, the maximum force the spindle can exert is generally so large that it can cause DNA breakage; in other words, the spindle alone can behave as a clastogenic factor (Friedberg, 1985). A noticeable exception is the budding yeast, where the spindle alone cannot break DNA. DNA breakage observed in this organism in the absence of topo II must involve other factors, such as an endonuclease.

Why is F_{max} so large?

Nicklas (1983) has concluded from the analysis of his force measurements that F_{max} is unexpectedly large. This conclusion relies on the assumption that in a normal anaphase, only the viscous resistance to movement exerted by the surroundings of the chromosome must be overcome. An estimate of the force F_{drag} required to overcome this viscous drag has been calculated by Taylor (1965) and Nicklas (1965), assuming a Newtonian behavior. The force is given by Stokes' law:

$$F_{drag} = \eta_{sol} S v, \quad (1)$$

where η_{sol} is the solvent viscosity, S describes the shape of the chromosome, and v its absolute velocity. For a relative velocity of 6 nm s^{-1} and a solvent viscosity of about 3 to 8 poises (Taylor, 1965; Alexander and Rieder, 1991), one obtains $F_{drag} \simeq 0.2-0.5$ pN. This force is therefore much smaller than F_{max}. However, the assumption that this viscous drag is the only resistance to movement to be overcome during anaphase is in reality only valid after the complete removal of the intertwinings between the sister chromatids. It is our contention that the large value of F_{max} can only be understood if one takes into consideration the fission process. This can be seen by looking at the cases where one chromosome must pass entirely through another one (Fig. 1, *C* and *E*). In these cases, where the fission process is successful, a significant stretching of the chromosomes is observed; the increase in length can reach 30%. This stretching is in fact close to the increase in length generally observed at the moment when chromosomes break in Fig. 1 *B* (about 30 to 40%; Bajer, 1963). If we assume that these chromosomes also behave as Hookean bodies, this means that the maximum force exerted by the spindle when one chromosome passes through another one can approach $F_{break} \approx 500$ pN. We conclude that there exist situations during anaphase in which the force exerted by the spindle not only greatly exceeds F_{drag} but also approaches F_{break}—the system operates close to its limit. One may argue that the biological relevance of these situations is limited, because they deal with exceptional variations from the standard anaphase. In the same spirit, Nicklas (1983) has observed that "the spindle's full capacity as a force producer sometimes comes into play in natural circumstances, most obviously when the chromosomes fail to separate properly in anaphase and are stretched by the spindle. In that circumstance, anyway, the adaptative significance of the capacity to produce relatively great force is not obvious and may be nil, since it may lead to chromosome breakage and genetically defective cells." We have seen here that this adaptive significance is not nil, because it permits the successful segregation of interlocked chromosomes. We further believe that situations in which one chromosome must pass entirely through another one are in reality much more frequent than is commonly realized:

1. In meiosis, chromosomes and bivalent interlocks resulting from crossing over or synapsis are frequently encountered in early prophase in a number of organisms (von Wettstein et al., 1984; Murray and Szostak, 1985; Rose and Holm, 1993). Rose and Holm (1993) have shown that there is a topo II meiosis-specific arrest in the budding yeast; they have proposed that this checkpoint has evolved to ensure the resolution of fortuitous interlockings between nonhomologous chromosomes. No such checkpoint is detected in mi-

tosis; we view the difference as representative of the much greater frequency of chromosomal interlockings in meiosis compared to mitosis in this organism.

2. This passage is also required in the case of chromosomes with diffuse kinetochores (holocentric chromosomes) arranged in a crossed configuration observed in *Luzula campestris* (wood rush) (Oestergreen, 1949; Mazia, 1961).

3. We also expect this passage to be required in the case of large enough chromosomes with a plectonemic coiling.

The reason that this passage event is considered infrequent lies most probably in the difficulty in explaining its mechanism (Darlington, 1937; Oestergreen, 1949; Darlington and Wylie, 1953; Mazia, 1961; Bajer, 1963).

On the other hand, we fully agree with Nicklas' proposal that the force generated by the spindle can play a crucial role in the circumstances leading to chromosome breakage and genetically defective cells. We believe that to understand several types of aneuploidy (such as nondisjunction) it is absolutely necessary to consider the fission process and the traction of the spindle during this fission. The remarkable fact that a normal anaphase can take place under conditions close to the physical limitations of the system (i.e., with a traction close to F_{break}) is likely to be of great significance if one wishes to explain failure in mitosis, and to be of even greater significance in meiosis.

To sum up, there exist situations in anaphase in which a force close to F_{break} is exerted by the spindle. They occur in particular when one chromosome passes entirely through another one. These situations have long been considered exceptional, but could in reality occur quite often, especially in the course of meiosis, when genetic recombination or synapsis frequently produces chromosomal interlocking. If the maximum force a spindle could exert were much smaller than F_{break}, the passage of one chromosome through another could ultimately take place, but their velocity would be much reduced. It seems reasonable to postulate that the motion of the chromosomes should be synchronous during anaphase, and that it is this requirement that has led to the evolutionary choice of a force F_{max} large enough to permit a constant velocity in anaphase under various situations.

Biological safety in anaphase

Given the fact that the force produced by the spindle can break a chromosome, how can the process of anaphase be devised to be as safe as possible? Let us consider the removal of the topological intertwinings between the sister chromatids by topo II in a standard anaphase (Duplantier et al., 1995a). The topo II first diffuses to a stress point appearing between the interlocked loops of the chromatids. It removes the stress point on a time scale k_{cat}^{-1} (where k_{cat} is the turnover number of topo II). This strand-passage reaction defines a local relative velocity $V_{topoII} = 2d \times k_{cat}$ (where $d = 2$ nm is the thickness of a double helix). Now, because a stress point submitted to the traction of the spindle is in danger of being mechanically broken, it is biologically safe to try to minimize the deformation of the DNA loops. Macroscopic deformations of the chromatids are certainly to be avoided, because the elastic constant of the chromatids can be close to the tensile strength of DNA. The safest way to handle this problem is to adjust the relative velocity V at which the two sister chromatids move apart below V_{topoII}; in this manner, the DNA strands can pass through one another without seeing each other (essentially with no deformation). This condition defines biological safety, a concept that we invoked in Duplantier et al. (1995a) and which we hope is now clarified. In addition, we have previously concluded that because of the relatively small values of k_{cat} (about 1 s^{-1} (Osheroff et al., 1983; Lindsley and Wang, 1993), but possibly 30 s^{-1} as discussed in Duplantier et al., 1995a), the velocity V has evolved to be roughly equal to V_{topoII}:

$$V \approx V_{topoII} = 2d \times k_{cat}, \tag{2}$$

a striking example of enzymatic perfection. A value of $k_{cat} \approx 30\ s^{-1}$ gives a velocity $V_{topoII} \approx 0.12\ \mu m\ s^{-1}$, which is indeed slightly greater than the highest relative velocity observed in anaphase (about 0.1 μms^{-1}). In conclusion, biological safety in anaphase can be obtained by adjusting the velocity V to V_{topoII} in such a manner that the stress points are relaxed almost immediately; this behavior is known in polymer physics as a phantom chain behavior. The concept of a phantom chain behavior made possible by topo II has previously been introduced in a conjecture on chromosome condensation (Sikorav and Jannink, 1994).

The large value of the maximum force produced by the spindle also allows consideration of the nature of the molecular glue that holds the sister chromatids together until the onset of anaphase. Before the start of anaphase, the metaphase chromosome is already submitted to the traction of the mitotic spindle (Nicklas, 1988). In fact, recent experiments indicate that tension from the mitotic forces is a prerequisite for the entry into anaphase (Li and Nicklas, 1995). What is the nature of the molecular glue that holds the sister chromatids together in spite of that tension? Murray and Szostak (1985) suggested that the residual DNA intertwinings between the chromatids perform this function of holding together the sister chromatids until anaphase. According to this hypothesis, the DNA intertwinings would therefore be submitted to the traction of the spindle; the tension would be only relaxed at the onset of anaphase by the intervention of topo II. In view of the large value of F_{max}, we think that this situation would be biologically unsafe. Therefore, we favor another hypothesis, according to which there exist linking proteins that are submitted to the traction of the spindle, and which are destroyed at the onset of anaphase (see the review on this problem by Miyazaki and Orr-Weaver, 1994).

Topo II as a motor during anaphase

Topo II dissipates energy during the strand-passage reaction; the enzymatic activity is coupled with ATP hydrolysis.

The number of ATP molecules hydrolyzed during a strand-passing event varies between 2 and 7 (Lindsley and Wang, 1993). Why ATP is hydrolyzed during the reaction is not obvious; the enzyme operates through a transesterification reaction that in principle does not require an energy source. In fact, topo I, the mechanism of which is similar to that of topo II, does not require an energy source (Wang, 1971). From the dynamical point of view, the dissipation of energy by topo II suggests that this protein could act as a motor. Indeed, the cleavage of one of the two DNA double helices at a stress point breaks the spatial symmetry, and according to Curie's principle, dissipation of energy in this asymmetric structure should act as a motor. The hydrolysis of two ATPs corresponds to about 9.9×10^{-20} J, and the dissipation of this energy over a length of 4 nm (twice the diameter of the double helix) corresponds to a force of about 25 pN. An (unknown) fraction of this energy could be transformed into a force acting on the DNA strands. It is possible that such a force plays a role in the fission process, in particular in the cases where a "repulsion" is observed between the sister chromatids in organisms when the mitotic spindle has been disrupted (Mazia, 1961; Bajer and Molè-Bajer, 1962). On the other hand, it is likely that a significant fraction of this energy is dissipated as heat. In this article we shall assume that most of the energy dissipates as heat and therefore neglect to a first approximation the possible role of topo II as a motor. Because before the breakage of one of the two double helices the structure formed by the crossing of the two double helices is symmetric, we shall view topo II activity as a random (Langevin) force associated with the strand-passing reaction.

CHROMOSOME NONDISJUNCTION IN THE ABSENCE OF TOPO II

In this section we consider dynamical models for the fission process in the absence of topo II. The fission may be thought of as a creep experiment, in which the sample is pulled apart at a constant velocity. In the absence of enzyme, the intertwined chromatids are expected to behave as a soft solid. To analyze the behavior of such solids submitted to the traction of the spindle, we must distinguish two basic types of chromosomes (Fig. 2). In the first type (Fig. 2 *A*; represented also in Spell and Holm, 1994) the kinetochore is not straddling DNA intertwinings between the two sister chromatids. This appears to be the case in the budding yeast; the experiments of Spell and Holm (1994) show that short enough linear chromosomes can be separated in the absence of topo II. Furthermore, there is a single microtubule attached to the kinetochore, and the DNA centrometric sequence is very small (about 110 bp) (Clarke and Carbon, 1985). In the second type of structure (Fig. 2 *B*; represented also in Brinkley et al., 1989) the chromosome is folded into radial loops stemming from a scaffold, and the kinetochore is straddling several intertwinings between the loops of the two sister chromatids. The chromatids are therefore topologically catenated and cannot be separated in the absence of enzyme. We expect this situation to be the usual one in the standard anaphase of large chromosomes analyzed previously (Duplantier et al., 1995a). We propose below two dynamical models for these two types of chromosomes.

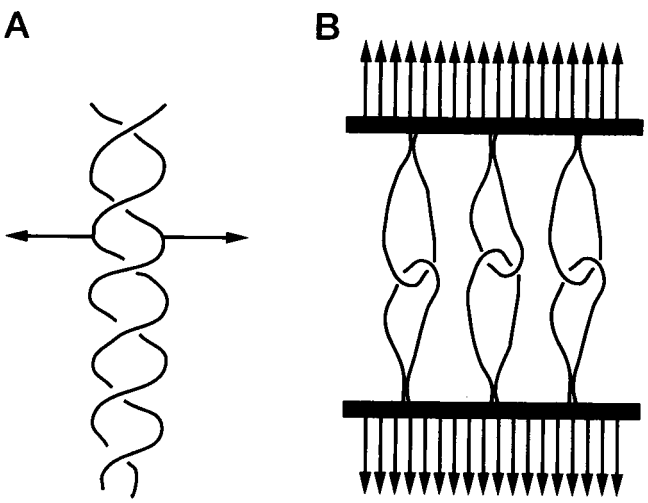

FIGURE 2 Schematic representation of the two basic types of chromosomes during anaphase. (*A*) Anaphase in the budding yeast according to Spell and Holm (1994). Each chromosomal spindle fiber is made up of a single microtubule (arrow), and the kinetochore is not straddling intertwinings between the sister chromatids. There exist on average $N_e \approx 20$ nucleosomes between two intertwinings (appearing as crossings on the figure). (*B*) Standard anaphase of large chromosomes. The chromatids possess a radial loop organization. DNA loops of the two chromatids are catenated. Each chromosomal spindle fiber is made up of several microtubules (arrows), and the kinetochores (black rectangles) are straddling several intertwinings between the loops of the sister chromatids.

Anaphase in the budding yeast

Spell and Holm (1994) have carried out a detailed analysis of the behavior of yeast chromosomes in mutants lacking topo II. Their study shows that the behaviors of small and large chromosomes differ. Small chromosomes (380 kb or smaller) can segregate successfully in topo II mutants, but large chromosomes are broken (note that the breakage is not due to the action of the spindle alone, as explained above). The observation that the small chromosomes can segregate shows us that we are dealing here with the first type of chromosome (Fig. 2 *A*), as discussed above. The critical chromosomal length above which the spindle becomes inefficient has been investigated by using normal and rearranged chromosomes III; the normal chromosome III used in their study is a metacentric one with two arms of approximately 100 and 250 kb. This chromosome is not broken during anaphase. In contrast, breakage in anaphase is observed for a rearranged chromosome III that is telocentric, with a long arm of 320 kb. Thus, although this rearranged chromosome is only 320 kb long, an increase in the size of the long arm from 250 kb to 320 kb has caused breakage.

We propose below an analysis of the behavior of the chromatids submitted to the traction of the spindle.

Chromosomal segregation in the absence of spindle traction

We consider first the problem of the segregation of the intertwined sister chromatids in a cell lacking both topo II and mitotic spindle. As done previously (Sikorav and Jannink, 1994), we treat the n-base pair chromatid as a flexible homopolymer, the monomer of which is a nucleosome. The radius of the nucleosome is $a \approx 5.5$ nm, and the degree of polymerization of the chromatid is $N = n/200$ (200 base pairs per nucleosome). Let us evaluate the characteristic number of base pairs below which the sister chromatids could separate by Brownian motion. As a result of the intertwinings between the sister chromatids, the chains will have to undergo a motion akin to reptation, and the duration of this process will be a reptation time equal to (de Gennes, 1979)

$$\tau_{rep} = \tau_{el} \frac{N^3}{N_e}, \tag{3}$$

where τ_{el} is the characteristic time associated with the motion of a monomer (a nucleosome), and N_e is the average number of monomers between two entanglements along the chromatids. Notice that the reptation model requires Gaussian statistics for the chains. In our situation, we deal with two intertwined sister chromatids, the level of condensation of which appears to be similar to that of eukaryotic interphase DNA (Guacci et al., 1994). Van den Engh et al. (1992) have shown that interphasic DNA possesses a Gaussian structure between 0.1 and 2 megabases. Their finding makes plausible our assumption that the intertwined chromatids considered here are Gaussian, and that the reptation model is valid.

In the case of sister chromatids, the entanglements are expected to result from the replication process. Because topo II activity is lacking, the entanglements should appear at the termination of the replication units (Sundin and Varshavski, 1981; Duplantier et al., 1995a), where about 10 interlocks should be created. In the budding yeast, the average distance between DNA replication origins is about 40 kb long (Kornberg and Baker, 1992), or 200 nucleosomes. This leads to a typical value for N_e of about 20. As a rough estimate for τ_{el}, we use the typical relaxation time associated with a Brownian sphere of radius $a = 5.5$ nm: $\tau_{el} = 4\pi\eta_{sol}a^3/k_BT$. Using as before $\eta_{sol} = 3$ poises for the cytoplasmic viscosity, we obtain $\tau_{el} \approx 1.5 \times 10^{-4}$ s, and

$$\tau_{rep} \approx 7.5 \times 10^{-6} N^3 \text{ s}. \tag{4}$$

If we allow the process to last for a plausible time of about 10^3 s, we obtain a value of 510 for N, corresponding to about 10^5 base pairs. This computation shows that the traction of the spindle is strictly required for long enough chromosomal chains (of more than 10^5 base pairs). We note that the value obtained for N is proportional to the cubic root of the parameters τ_{el} and N_e. This results from the reptation model and should make the computation robust. Indeed, a reptation process has previously been considered for the process of chromosome condensation (Sikorav and Jannink, 1994), and similar values of N have been obtained (compare Eq. 4 here with equation 14 in Sikorav and Jannink, 1994).

Chromosomal segregation in the presence of the mitotic spindle

Let us now evaluate the characteristic number n_c of bases below which the force applied by the spindle is able to separate the sister chromatids. In the fission yeast, where this process could be visualized, one can see thin protrusions (which contain the centromeres) extending from either side of the main chromatin mass, which does not separate (Funabiki et al., 1993). We conclude that in the absence of topo II, the two sister chromatids pulled by the spindle become entangled one with the other, and probably also with the surrounding replicated chromosomes. Because each chromosomal chain involved in the process is held by a spindle fiber, the reptation type of motion considered above is now made impossible. Physically, this process appears similar to the pullout of a tethered chain from a network (such as a rubber or a gel). Basically, we want to estimate the friction coefficient $\zeta(n)$ associated with the pullout of sister chromatids that are n base pairs long. In the smooth segregation regime, one expects the simple relation

$$\zeta(n) = \frac{F}{V}, \tag{5}$$

where V is the velocity of separation (17 nm s^{-1}; Palmer et al., 1989) and F is the (unknown) force exerted by the spindle (at most 47 pN). The models proposed for chain pullout reveal a complex situation, however, in which the chain friction coefficient depends on the pullout speed as well as the length of the chain (Rubinstein et al., 1993; Krupenkin and Taylor, 1995). We shall recall the results obtained by Rubinstein et al. (1993).

These authors consider the motion of a long tethered chain (made of N monomers), pulled with a constant velocity V out of a rubber made of chemically identical chains and characterized by a mesh size $D_e = N_e^{1/2}a$ (a being the size of a monomer). The tethered chain is assumed to be entangled with the rubber, the diameter of the Edwards' tube (Doi and Edwards, 1986) being equal to D_e. A characteristic Brownian force f^* associated with the fluctuations within this tube is defined by

$$f^* = \frac{k_BT}{D_e}. \tag{6}$$

When the force F exerted on the tethered chain is below f^*, the chain is undeformed. Above f^* the pulled chain begins to extend. Three velocity regimes can be distinguished:

• At very low velocities (corresponding to $F < f^*$) the chain friction coefficient is proportional to the relaxation time τ_N of an arm of a star polymer: $\tau_N = \tau_{el} N^2 \exp(\mu N/N_e)$, where μ is a numerical constant ($\mu \approx 15/8$). This regime ends for

$$V > V_1 \cong \frac{D_e}{\tau_N}. \tag{7}$$

• At very high velocities, the chain is completely stretched and disentangled, and the friction is weak (Rouse like): $\zeta(n) \approx \zeta_{el} N$, where ζ_{el} is the elementary friction coefficient of a monomer. This regime requires

$$V > V_3 \cong \frac{D_e}{\tau_{el} N_e^2}. \tag{8}$$

• At moderate velocities ($V_1 < V < V_3$), the head region (near the tether) is strongly aligned. The tail forms a plume (made of k monomers) which is relaxed. The force exerted on the chain is given by

$$F = f^* + (N - k)\zeta_{el} V. \tag{9}$$

The force described in Eq. 9 is the sum of the force f^* exerted on the plume and of the Rouse friction force exerted on the $N - k$ monomers of the stretched portion. The length of the tail depends on the velocity V: for $V < V_2 = V_3(N_e/N)^2$, k is obtained by the condition $D_e/V = \tau_{el} \exp(\mu k^2/N_e N)$, whereas for $V > V_2$, the condition is

$$D_e/V = k^4 N_e^{-2} \tau_{el}. \tag{10}$$

This theoretical analysis may appear somehow intricate. We tentatively apply it to the pullout of a chromatid. We can compute f^* using $N_e \approx 20$ and $a = 5.5$ nm; Eq. 6 yields $f^* \approx 0.2$ pN. This is a very small force, which can be easily produced by the traction of the spindle. This means that the spindle is able to stretch the chromatid, provided that V is greater than V_1. Using Eq. 7 and $V = 17$ nm s^{-1}, one can see that V is equal to V_1 for a very small value of N ($N \approx N_e$). Because we deal with very long chains ($N \geq 10^3$), the condition $V > V_1$ is fulfilled, and the chains should be stretched; this result agrees with the observations made in the fission yeast (Funabiki et al., 1993). On the other hand, the value of V_3 calculated with Eq. 8 is equal to about 400 nm s^{-1} (using $\tau_{el} \approx 1.5 \times 10^{-4}$ s as above). We are therefore below V_3, but (for $N > 100$) above $V_2 = V_3(N_e/N)^2$. Accordingly, the size of the plume is given by Eq. 10, and this yields a small value for k of about 40 monomers.

The picture provided by this analysis is therefore that of an almost completely extended chain, with a very small plume. Using $\zeta_{el} = 6\pi\eta_{sol}a$, the force predicted by Eq. 9 becomes

$$F \approx f^* + N\zeta_{el} V = f^* + 6\pi\eta_{sol} aNV. \tag{11}$$

This equation differs slightly from Eq. 5 by the existence of the threshold force f^*. The second term on the right-hand of Eq. 11 is equal to f^* for $N \approx 300$. For $N > 300$, the friction becomes essentially Rouse-like (i.e., proportional to N).

To use Eq. 11, we must take into account the beadlike structure of the chromosomal fiber. Indeed, when a chromatid is submitted to a given traction F, the typical Brownian excursion under such tension, d, is given by $d = k_B T/F$. When the distance d becomes smaller than the radius of a nucleosome, tight entanglements of nucleosomes appear that can no longer be suppressed by thermal fluctuations. Their suppression would necessitate the intervention of topo II. This introduces a second characteristic force:

$$f^{**} = \frac{k_B T}{a} \approx 0.8 \text{ pN}. \tag{12}$$

The force f^{**} corresponds to the appearance of tight entanglements of the pulled-out chromatid with the surrounding chromosomes, and these should stop the pullout process. A similar reasoning has already been proposed in the study of electrophoresis in strong fields to account for the trapping of charged polymers in metastable configurations (Olvera de la Cruz et al., 1986; see also Viovy and Duke, 1994). In such circumstances, when the force F exceeds f^{**}, one expects the time τ required for the pullout of a chain of M monomers to grow exponentially fast as

$$\tau \propto \exp\left(cM \frac{F}{f^{**}}\right) \tag{13}$$

where c is a numerical factor (Olvera de la Cruz et al., 1986). This time corresponds to the crossing of M successive Kramers energetic barriers. Such a relation ensures that the pullout process is completely stopped. In our case, the number of barriers M should correspond to the number of tight entanglements multiplied by the number of nucleosomes that must slip over them. The force f^{**} therefore provides a physical upper bound for the disentanglement process.

The pullout is thus stalled when F in Eq. 11 is equal to f^{**}. Numerically one obtains $N_c \approx 1100$, corresponding to $n_c \approx 220{,}000$ base pairs. The agreement with the experimental data of Spell and Holm (1994) is fair.

This conclusion should be considered with some care, however, for several reasons:

1. We have supposed that the biological process under examination is equivalent to that of a chain pulled out of a permanent network (a rubber). Yet it is not obvious that the sister chromatid and the surrounding chromosomes can be considered as a physical realization of such a network.

2. The description of the several regimes in this model is rather complex (Eqs. 7–10).

To these two criticisms, one may answer that in spite of the complexity of this model, its use here boils down to the computation of an elementary Rouse friction (proportional to N; Eq. 11). This friction interpolates from the force f^* (Eq. 6) to the force f^{**} (Eq. 12). Both of these Brownian forces are characteristic forces in our system and do not depend on the model of Rubinstein et al. (1993). Their

consideration here appears rather robust. That the force f^{**} provides us with an upper bound results from the exponential behavior of Eq. 13.

3. Last, the viscosity η_{sol} plays a role in the final evaluation of N_c, because the stretched part of the chain is assumed to be immersed in the cytoplasmic medium. In reality, the surrounding chromosomal chains could increase this solvent viscosity. Such an effect could tentatively be taken into account by using the effective medium theory (equation 5.190 in Doi and Edwards, 1986), as done previously by Sikorav and Jannink (1994). Here the chain concentration is smaller (by a factor of about 5) than the concentration of a nucleosome melt considered in that work. Consequently, the effective viscosity would be greater than the solvent viscosity by a factor of 3 at most. Even in this extreme case, the order of magnitude obtained for N_c would remain the same.

It should also be noted that Krupenkin and Taylor (1995) have recently proposed a microscopic theory of the pullout process in which the pullout force can scale as N^3 for $N \gg N_e$. Unfortunately, their description involves several unknown microscopic parameters, and it is therefore difficult to apply their results to the problem considered here.

It is historically instructive to mention that the chain pullout model discussed here is reminiscent of a mechanism discussed by Watson and Crick (1953) for the separation of the complementary strands of the DNA double helix, in which "one takes hold of one end of one chain and the other end of the other, and simply pulls in the axial direction. The two chains slip over each other, and finish up separate and end to end."

To sum up, we have discussed two models for the segregation of yeast chromosome in the absence of topo II: 1) When the spindle traction is also missing, a reptation model shows that only short chromosomal chains (smaller than about 10^5 base pairs) can separate in a reasonable time. 2) In the presence of the mitotic spindle, the segregation can be described by a chain pullout model; this model predicts that the chromosomal chains should be smaller than about 220,000 base pairs to be successfully segregated. The agreement with the experimental data of Spell and Holm (1994) is fair.

Standard anaphase in the absence of enzyme

In a standard anaphase, we expect the kinetochore to be large enough to straddle numerous intertwinings of the chromosomal loops of the sister chromatids. The problem of the mechanical separation of the chromatids in the absence of enzyme is then similar to the mechanical rupture of polymeric rubbers under elongational strain, for which a model has been proposed by Lake and Thomas (1967). In the rupture of rubbers, the significant quantity is the energy T of rupture per unit area on which the stress applies. For rubbers T is found to have a typical value $T_o = 50$ J/m^2. Here we must rederive T for the particular case of the chromatid intertwinings. In the tearing of rubbers the equation giving T is

$$T = \frac{C}{S}\tilde{n}u, \tag{14}$$

where C is the number of connectors (effective polymeric chain) per surface S, $\tilde{n}$ is the number of monomers in such connectors, and u is the binding energy of a monomer. Equation 14 indicates that the tearing energy can be much greater than the dissociation energy of a monomer u. This results from the hypothesis that to rupture a connector chain made of $\tilde{n}$ monomers, an energy equal to $\tilde{n}u$ is required, although only one of the monomer units will in fact be ruptured (Lake and Thomas, 1967).

Experiments of the sonic degradation of DNA show that chain scission predominantly occurs with a C-O bond rupture (Richards and Boyer, 1965). For this reason, we shall take as an estimate for u twice the C-O bond energy (Israelachvili, 1985):

$$u = 1.13 \times 10^{-18}\ \text{J}. \tag{15}$$

To compute T for the two sister chromatids, we consider the simple geometrical model of a human metaphase chromosome proposed earlier (Duplantier et al., 1995a). In this model the sister chromatids are described as overlapping cylinders. In this situation a connector is defined by an intertwining of two chromosomal loops. The exact organization of the chromosomal DNA chains in the centromeric region is unknown; we shall assume here that it is similar to that of an R band (Saitoh and Laemmli, 1994). We note that this constitutes a very crude description: the centromeric constriction contains large amounts of heterochromatin, the particular structure of which is most likely directly involved in the cohesion of the sister chromatids (Miyazaki and Orr-Weaver, 1994). The corresponding linear density of intertwinings between the sister chromatids is about 300 μm^{-1} along their long axis. Now the structure of a human kinetochore is roughly cylindrical, with a diameter of about 0.2 μm. There should be about 60 such connectors beneath the kinetochore (0.2 μm $\times$ 300 μm^{-1}).

Describing again the chromosomal loop as a chain of nucleosomes, we take $\tilde{n} = 300$ nucleosomes (for a loop of about 60 kb).

Using for the surface of the kinetochore $S = \pi \times (0.1\ \mu\text{m})^2$, we obtain for T

$$T = 0.64\ \text{J/m}^2, \tag{16}$$

a value much smaller than the tearing energy of rubbers. In spite of the crudity of our model, we believe that the value obtained for T is plausible.

We now compare this energy T with the available power per unit area provided by the mitotic spindle:

$$\frac{P}{S} = \frac{F_{max}}{S}V. \tag{17}$$

Because a typical human spindle contains about 15 microtubules (Rieder, 1982), we take $F_{max} = 700$ pN. A typical value for V is 30 nm s^{-1} and hence

$$\frac{P}{S} = 6.7 \times 10^{-4}\ \mathrm{W/m^2}. \quad (18)$$

The time t_r required for a mechanical rupture is

$$t_r = \frac{T}{[P/S]} = 960\ \mathrm{s}. \quad (19)$$

The time t_r is found to be greater than an upper limit (of about 10 min) for the duration of anaphase (Mazia, 1961). It is also worth noting that the distance traveled during the time t_r is about 30 nm $s^{-1} \times 960$ s, or 30 μm; it is roughly consistent with a deformation of a 60-kb Laemmli's loop yielding a fully extended DNA loop. We conclude that in the absence of topo II, the cell is likely to leave anaphase within a time too short to allow the mechanical breakage of the intertwinings. This conclusion provides a tentative physical explanation for the nondisjunction observed in such situations.

The separation process in the presence of topo II may be viewed as an enzymatically assisted rupture. The enzyme allows the chains to cross each other at each entanglement, thereby reducing stress. The energy that is now dissipated corresponds to the pullout process of the connector chains. It is difficult to calculate the energy for the situation of the sister chromatids. In the case of the separation of polymeric chains taking place in vacuo, the pullout process would be associated with a van der Waals energy U_w, and the new enzyme-assisted "tearing" energy would be obtained by substituting U_w for u in Eq. 14. Because U_w is much smaller than u (by a factor of 50), the time t_r associated with U_w in Eq. 19 would now be compatible with the duration of anaphase. This estimate is very rough, however. The force required to pull apart the chromatids in the presence of topo II could be much smaller than the force F_{max} giving the time t_r; in addition, it does not take into account the energy available from ATP hydrolysis during the strand-passage reaction, which is precisely of the order of U_w. We shall therefore consider in the next section a different point of view to analyze the dynamics of the chromosomes in the presence of topo II.

Spindle traction and apoptosis

Our analysis of the action of the spindle on chromosomal DNA indicates that it can act as a clastogenic compound, i.e., it introduces breaks in the DNA molecule, either alone or in conjunction with endonucleases. This leads us to propose that the spindle could be involved in some metabolic pathways, leading to an apoptotic death of the cell. This proposal is buttressed by the following arguments: 1) First, it is already known that DNA damage can trigger apoptotic cell death; an example is provided by the experiments on the irradiation of G_1 cells. The DNA strand breaks produced are sufficient to induce apoptosis (Nelson and Kastan, 1994). 2) Apoptotic cell death induced by various compounds (including topo II inhibitors) is often accompanied by the formation of large DNA fragments 50 to 300 kb in length (Walker et al., 1991; Brown et al., 1993; Oberhammer et al., 1993; Cohen et al., 1994; Beere et al., 1995). It was proposed that this pattern of fragmentation reflects the loop organization of the chromosomal fiber (Filipski et al., 1990; Brown et al., 1993; Lagarkova et al., 1995). According to our proposal, this fragmentation pattern could involve the traction of the spindle on DNA loops, acting in conjunction with endonucleases. It will be of interest to determine whether the fragmentation pattern is modified if drugs preventing the formation of the spindle are also present within the cells.

CHROMOSOMAL MOBILITY IN THE PRESENCE OF TOPO II

In this section we discuss the coupling between DNA dynamics and topo II activity. We first describe a simple model and then show how this model can be applied to the special case of the passage of one chromosome through another during anaphase.

A diffusion process associated with topo II strand-passage reaction

Type II DNA topoisomerases are known to be active even in the absence of the spindle force; they can relax supercoiled DNA molecules or drive catenation/decatenation reactions. In such cases, thermal fluctuations induce collisions between DNA double helices, which are transformed by catalysis into DNA strand-passing events. With each strand-passing event we can associate a microscopic relative displacement $\delta x = \pm 2d$, where d is the diameter of the double helix. The plus or minus sign expresses the fact that the double helix located on one side of the binary cluster moves across the other double helix, or vice versa.

With this local random displacement process we associate an equivalent diffusion coefficient in one dimension, D, which describes a model Brownian motion of a DNA chain through a series of closely packed DNA obstacles in the presence of topo II (Fig. 3). Because the strand-passage reaction requires a time τ_{cross}, we have by definition $(\delta x)^2 = 2\,D\tau_{cross}$, or

$$D = \frac{1}{2}(2d)^2 k_{cat}. \quad (20)$$

For $d = 2$ nm and a typical value $k_{cat} \approx 1\ s^{-1}$, we obtain $D \approx 8\ \mathrm{nm^2\ s^{-1}}$. We now introduce a friction associated with this diffusion coefficient using Einstein's relation, which is a particular case of the fluctuation-dissipation theorem (van

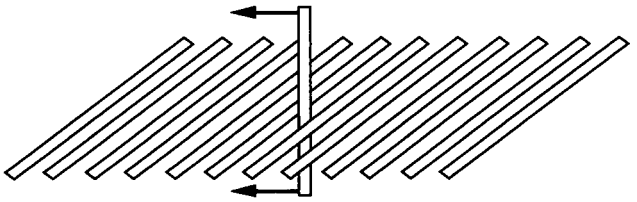

FIGURE 3 Motion of a DNA chain through a series of closely packed (2 nm apart) DNA obstacles in the presence of topo II. The strand-passage reaction permits a one-dimensional diffusion process characterized by the diffusion coefficient D of Eq. 20. When a small force F_{pull} (arrows) is exerted on the chain, the chain moves with a uniform velocity, given by Eq. 23.

Kampen, 1992):

$$\zeta = \frac{k_B T}{D}. \tag{21}$$

We obtain at room temperature $\zeta \approx 0.5$ g s^{-1}. This friction ζ is huge in comparison with classical examples; it can be thought of as an effective protein friction due to the strand-passing reaction.

The reasoning developed here is similar to an analysis of the one-dimensional diffusion of microtubules bound to dynein (Vale et al., 1989; Tawada and Sekimoto, 1991; Sekimoto and Tawada, 1995). In that system, microtubules bound to immobilized dyneins perform a one-dimensional random walk characterized by a diffusion coefficient $D \approx 10^4$ nm^2 s^{-1}, from which a frictional coefficient $\zeta \approx 10^{-4}$ g s^{-1} can be calculated (using Eq.21). This coefficient is typically two orders of magnitude larger than the expected hydrodynamic friction coefficient and is understood as a protein friction coefficient (Tawada and Sekimoto, 1991; Sekimoto and Tawada, 1995). [We note here that there is a difficulty associated with this description; it is in general possible to express ζ as the product of a mass M times a characteristic frequency of the system τ^{-1}: $\zeta = M \times \tau^{-1}$. Such a relation leads to consistent estimates for M in the case of Brownian motion in simple liquids (Hansen and McDonald, 1986), but in our case we would take as the characteristic frequency $k_{cat} \approx 1$ s^{-1}, which is very small. Because ζ is huge, this leads to a mass $M \approx 0.5$ g, which has no physical meaning. The same problem is encountered in the system microtubule/dynein: the characteristic frequency (the rate constant of the binding/unbinding reaction) is typically 10^3 s^{-1} and the corresponding mass $M \approx 10^{-7}$ g, which is much larger than the mass of the diffusing microtubule (about 10^{-15} g).]

Let us now exert a small constant force F_{pull} on the Brownian DNA chain of Fig. 3. As a result of the action of the enzyme, the external force can now be dissipated by the friction derived above according to the fluctuation-dissipation theorem. The chain will move with a uniform velocity V given by

$$V = \frac{F_{pull}}{\zeta}, \tag{22}$$

where the friction coefficient is given by Eq. 21. Combining with Eq. 20 we obtain

$$F_{pull} = \frac{k_B T V}{2d^2 k_{cat}}. \tag{23}$$

Equation 23 tells us that the force F_{pull} required to move a DNA chain in a series of closely packed DNA obstacles at a constant velocity V is inversely proportional to k_{cat}. It is of interest to examine the particular case corresponding to "catalytic perfection": $V = V_{topoII} = 2d \times k_{cat}$ (Eq. 2). It leads to the simplified relation

$$F_{pull} = \frac{k_B T}{2d} \approx 1 \text{ pN}. \tag{24}$$

The force required to move the chain would then simply be the thermal energy divided by the thickness of the double helix. Furthermore, in this particular case the energy associated with a single strand-passage reaction is simply

$$E = F_{pull} \times 2d = k_B T. \tag{25}$$

The force computed in Eq. 24, $F_{pull} \approx 1$ pN, shows that catalytic perfection is compatible with biological safety. The force exerted on the DNA chains is much smaller than F_{break}; it is also much smaller than the force $F_{stretch} \approx 70$ pN required to stretch the DNA molecule beyond its crystallographic length (Cluzel et al., 1996; Smith et al., 1996).

It is also of interest to compare the energy associated in such a model with a strand-passage event, $E = 4.1 \times 10^{-21}$ J (Eq. 25), with the energy required for the mechanical breakage of DNA (Eq. 15); $u = 1.13 \times 10^{-18}$ J. Under the condition of catalytic perfection, the energy required for the topo II-assisted strand passage of 60 connectors could be reduced to 60 $k_B T$ instead of a total energy $C\tilde{n}u = 8 \times 10^4 k_B T$ in the absence of enzyme according to Eq. 14. However, in a standard anaphase, the interlocks present at the periphery of the cylindrical sister chromatids are assumed to be removed in a very small amount of time, rather than by a series of small jumps, as in the model examined here. We believe that the fluctuation-dissipation relation established above (Eq. 23) applies better to the situation described below.

Passage of one chromosome through another one using the fluctuation-dissipation theorem

The fluctuation-dissipation relation (Eq. 23) corresponds to a succession of strand-passing events. Biologically, this situation is encountered when one chromosome passes entirely through another one. We saw previously (see discussion after Eq. 1) that in that particular case, F is constant and close to $F_{break} \approx 500$ pN during the passage, and that the velocity V remains constant. We can therefore define a macroscopic friction coefficient $\zeta_{macro} = F_{break}/V$.

We consider here the passage of two human chromosomes through one another. For convenience, we shall assume that the passage takes place at R bands (where the

loop structure is simpler) for both chromosomes (Fig. 4). A typical value for V is 30 nm s^{-1}, leading to $\zeta_{macro} \approx 17$ g s^{-1}. It is clear that ζ_{macro} is so large that it cannot be accounted for by the hydrodynamic friction due to the chromatid loops. Our contention is that this macroscopic friction results from the summation of the microscopic protein friction associated with each topo II molecule involved in the process.

According to our previous analysis (Duplantier et al., 1995a), the number of topo II molecules locally available for the removal of the entanglements is $n_0 \times L$, where $n_0 = 450\ \mu m^{-1}$ is the number of available topo II per μm, and $L = 0.8\ \mu$m is the height of the chromatid segment involved in the passage event. The duration of the passage is $2L/V \approx 53$ s, and during the passage time the enzymes can catalyze $2n_0 \times L \times L/V \times k_{cat}$ strand-passing reactions. If we make the assumption of catalytic perfection (Eq. 2), we can obtain a lower estimate for k_{cat} ($\sim 7.5\ s^{-1}$), leading to a number of about 1.4×10^5 available strand-passage reactions.

On the other hand, we can make a rough estimate of the number of entanglements to be removed during the passage, assuming that the two chromosomes are not deformed (Fig. 4). In our model, we have L/e minibands, where $e = 0.03\ \mu$m is the thickness of a miniband, and each miniband contains $b = 18$ radial loops. The number of entanglements to be removed is thus about $(Lb/e)^2$ or 2.3×10^5. This number is (slightly) greater than the number of available catalytic events! The number of catalytic events could be sufficient for a value of k_{cat} of 12 s^{-1} or greater, which is a high but not unrealistic value. We think that this result agrees with the well-known instability of circular or ring chromosomes. In Fig. 1 *D*, for instance, we see that when there is more than one interlocking between the two daughter chromosomes, the crossing fails. In this respect, it is also noteworthy that an unstable circular chromosome in the fission yeast can be stabilized by an overexpression of topo II (Murakami et al., 1995).

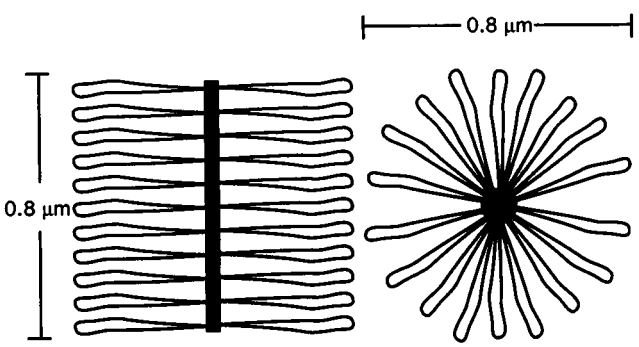

FIGURE 4 Schematic representation of two human chromosomes about to pass through one another. The two chromosomes are displayed with the radial loop organization of an R band (Saitoh and Laemmli, 1994; Duplantier et al., 1995a). The loops stem from the scaffold shown here as a black rectangle (*left*) or a black disk (*right*). The long axes of the two chromosomes are assumed to be perpendicular. The chromatids are organized in densely packed minibands (30 nm thick). Eleven such minibands are shown unstacked on the left chromosome (in reality there should be about 27 such minibands for a height of 0.8 μm). There are 18 radial loops in each miniband, which are shown for a single miniband on the right chromosome.

We observe, however, that our estimate of the number of entanglements to be removed relies on the assumption that the chromosomes are not deformed. In reality, because the force F is close to F_{break}, the chromosomes should be deformed, and the deformation will decrease the number of entanglements. We therefore adopt the point of view that at any time during chromosome passage, all of the topo II molecules of the R bands should be active and perform strand-passing reactions. In other words, at a given time, all of the entanglements under stress are being removed by the topo II, and their number is simply on the order of the number of available topo II (about 360 molecules). This agrees with the constant velocity observed during the crossing; an excess of entanglements under stress would presumably lead to a decreased velocity. The force exerted on each active entanglement is then simply $F_{max}/360 \approx 1.4$ pN. As previously, we note that this force is compatible with biological safety. Furthermore, this force is also very close to the force predicted by Eq. 24. In fact, Eq. 23 is satisfied for $F_{pull} = 1.4$ pN and $V = 30$ nm s^{-1} for a value of $k_{cat} \approx 11\ s^{-1}$, which is very close to that predicted by "enzymatic perfection." We conclude that the chromosome passage reaction is reasonably described by the fluctuation-dissipation theorem.

CONCLUDING REMARKS AND SUMMARY

Several physical problems result from the traction of the spindle exerted on chromosomal DNA during anaphase. A first problem, which has been discussed by Taylor (1965) and Nicklas (1965, 1983, 1988), deals with the traction of the spindle *after* the fission of the metaphase chromosome. This problem has been analyzed by these authors in the case where the two daughter chromosomes are no longer topologically linked. The force required to drag the chromosomes can then be estimated using Stokes'law and is found to be nearly 10,000 times smaller than the maximum force F_{max} the spindle can exert (Nicklas, 1983). This prompted us to look for other explanations for the large value of F_{max}. To do so, we have investigated the alternative problem, which is associated with the traction of the spindle during the fission process itself. We summarize here the main ideas and conclusions of the present work:

1. Because the sister chromatids are generally topologically intertwined at the onset of anaphase, there are in fact stringent constraints on the fission process. Thus, although the fission process itself occupies a small fraction of the duration of anaphase, we expect that an analysis of these initial constraints will lead to a better understanding of the entire mechanism of anaphase. This idea was introduced in a previous work (Duplantier et al., 1995a), in which we examined the kinematics of the removal of the topological constraints by type II DNA topoisomerases. In that case, the

constraints have apparently dictated the slow velocity of the chromosomes, even after the removal of the intertwinings. Here we have tried to extend this reasoning to the dynamics of the fission process.

2. The existence of topological intertwinings raises in particular the possibility of a breakage of the chromosomal DNA chain by the spindle. We have found that the maximum force the spindle can exert is in general greater than the tensile strength of DNA. This force is also probably large enough to be responsible for the breakage of two-kinetochore chromosomes. Furthermore, the maximum available power, $P = F_{max} \times V$ is typically 2×10^{-17} J s^{-1} in a mammalian cell (F_{max} = 700 pN, V = 30 nm s^{-1}) and can lead to the breakage of a DNA chain in a fraction of a second, according to the value of the energy given by Eq. 15. We have examined some implications of this finding for the process of anaphase; in particular, biological safety requires that DNA stress points resulting from the traction of the spindle be relaxed almost immediately by the intervention of topo II. This suggests an interpretation of the adjustment of the velocity of the chromosomes to V_{topoII} (Eq. 2).

3. We have seen that there exist peculiar physiological situations in which a force close to the force F_{break} required to break a chromosome must be exerted by the spindle. Such forced situations occur when one chromosome must pass entirely through another one. This illustrates that the spindle's full capacity as a force producer can be better understood by taking into consideration its action during the fission process itself.

4. In the absence of topo II, the behavior of the sister chromatids has been analyzed with two models: a) In the budding yeast, the segregation of linear chromosomes is permitted for small enough chains (Spell and Holm, 1994). We have tentatively explained these observations with an extrusion model (Rubinstein et al., 1993). b) In a standard anaphase, the absence of topo II leads to the nondisjunction of the metaphase chromosome. We have interpreted this phenomenon using the theory proposed by Lake and Thomas (1967) to calculate the tearing energy of rubbers.

5. We have analyzed the coupling that takes place between the enzymatic activity of topo II and the traction exerted by the spindle using the fluctuation-dissipation theorem. This leads to consistent relations between F_{pull}, V, and k_{cat} in a model system (Eq. 23) and can be used to describe the passage, mentioned above, of one chromosome through another. This description is very crude, however, and we hope to improve it in the future.

Possible experiments

It should be possible to test the analyses developed here in two different ways: first, through the micromanipulation of chromosomes, either in vivo (Nicklas, 1988; Liang et al., 1993) or in vitro; second, by using model systems of entangled DNA or nucleosomal chains (Perkins et al., 1994; Musti et al., 1995; Wirtz, 1995). In what follows, we suggest some possible experiments that could test these analyses.

We have seen that F_{break} should exceed 480 pN; one could determine its experimental value through stretching experiments similar to those of Nicklas (1983). This could require the use of two needles attached at the ends of the chromosome to generate sufficient stretching.

Similarly, one could determine the tearing energy T required to disrupt a standard metaphase chromosome in the absence of topo II activity. These experiments could be performed in vivo in cells in which the spindle has been disrupted, or in vitro using isolated metaphase chromosome. The calibrated traction would again be provided by needles. Several variants of this experiment can be thought of: one could determine T for the centromeric region, and for other regions of the arms of the chromosome, such as particular bands (Saitoh and Laemmli, 1994). The role of proteins supposed to be involved in the anaphase glue could also be tested.

In the presence of topo II activity, it would be of a great interest to know the maximum force actually exerted by the spindle during the fission process. In principle, it should be possible to determine the stress-strain relations during the fission process of either a standard metaphase chromosome or of two chromosomes passing entirely through one another. One could in particular check whether the maximum force exerted in the latter case is close to F_{break}. The catalytic role of topo II in the fission process could be studied in a systematic manner, either by modulating the quantity of available enzyme or by using inhibitors.

At the molecular level, the passage of one DNA double helix through another one in the presence of topo II could be studied by using artificially entangled DNA molecules, such as catenated rings submitted to an external traction. One could, for instance, vary the force exerted on the entanglement before the release of the topo II in the solution. This would make it possible to test whether a fluctuation-dissipation force of about 1 pN (Eq. 24) is indeed sufficient to drive the action of the enzyme.

Finally, chain pullout experiments with a calibrated extrusion force (Wirtz, 1995) could be performed on nucleosomal chains to check the existence of the threshold force f^{**} (Eq. 12) associated with the appearance of tight entanglements.

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