Kinetics of Chromosome Condensation in the Presence of Topoisomerases: A Phantom Chain Model

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ABSTRACT We discuss the requirement of type II DNA topoisomerase in the process of mitotic chromosome condensation. Using a known model describing the collapse of homopolymers, we propose that the compaction process necessitates a change in the topological state (i.e., a self-knotting) of the chromosomal chain. We argue that the enzymes are necessary to reach the compact metaphase state in a time interval that is much smaller than the time expected in the uncatalyzed process. The folding process is such that the potential entanglement points are localized at particular regions of the chromosome known as the scaffold-associated regions. The concentration of entanglements in the metaphase chromosome is related to the average size of the radial loops. A phantom chain model for the condensation process, in which each potential entanglement point is dealt with by a topoisomerase II molecule, is proposed.

INTRODUCTION

The dynamic properties of eukaryotic chromosomes can be observed in various cellular processes (for reviews see Alberts et al., 1990, and Herbomel, 1993). From the point view of polymer dynamics (Doi and Edwards, 1986), the understanding of these properties raises a problem whose general features appear to be the following:

1. Each chromosome contains a single molecule of linear double-stranded DNA. This fact has been established using different experimental approaches (including a viscoelastic method (Kavenoff and Zimm, 1973; Kavenoff et al., 1974) and physical mapping with pulse field gel electrophoresis (Schwartz and Cantor, 1984) and is assumed to hold true for all eukaryotic species. This general assumption is known as the unineme hypothesis. According to the unineme hypothesis, the degree of polymerization of chromosomal DNA ranges from 10^5 to more than 10^9 base pairs.

2. During the cell cycle, each chromosomal DNA is replicated, giving rise to a replicated chromosome composed of two sister chromatids. At the time of cell division, replicated chromosomes condense into individual structure called metaphase chromosomes, and each sister chromatid is then segregated to a daughter cell. The time scale for the cell cycle ranges from 10^3 to 10^5 s.

3. The relaxation time associated with the Brownian motion of a single DNA chain in solution would be of the same order of magnitude as the cell cycle time for a radius of gyration $R_G \approx 10^5$ Å. However, throughout the cell cycle, the polymer solution made up by the different chromosomes within the cell is non-dilute. Thus, the cellular processes occur under conditions where topological constraints are expected to slow down the motions of the DNA chains with respect to the Brownian motion in dilute solution.

The problem we consider here is that of chromosome condensation. This process takes place in 10^2 to 10^4 s and leads to a dense state with a peculiar structure. The problem of chromosome condensation is twofold: 1) How can one explain the time scale in which the process takes place? 2) What is the path that leads to the dense state? The condensation process is caused by the onset of attractive forces and is controlled by the interplay between repulsive and attractive forces. However, this is not sufficient and there is evidence that the effective condensation process is assisted by an enzymatic activity. Type II DNA topoisomerases (topo II) are known to be required both in vivo and in vitro to catalyze the late stages of the condensation process in mitosis (Newport and Spann, 1987; Uemura et al., 1987; Adachi et al., 1991; see Laemmli et al., 1992, for a recent review). Topo II catalyzes the concerted breakage and rejoining of both strands of a DNA double helix, allowing the passage of a second double-stranded DNA segment through the reversible transient break (Liu et al., 1980; Hsieh, 1990). These enzymes can alter the superhelicity in a circular DNA molecule and also form or resolve knotted and catenated structures. Topo II is also required for the proper segregation of the sister chromatids in anaphase (DiNardo et al., 1984; Holm et al., 1985; Uemura and Yanagida, 1986; Shamu and Murray, 1992).

Our goal is to interpret physically the requirement of topo II in the condensation process. For this, we use a model describing the kinetics of collapse of a homopolymer proposed by Grosberg et al. (1988a, b). In this model, the late stage of the collapse requires self-knotting of the chain; it allows to estimate the time required for chromosome condensation in the absence of enzyme. It is a reptation time that can overestimate the actual time by several orders of magnitude. This explains the requirement for the catalytic properties of the enzyme.

Conventionally, in polymer physics, a chain in which self-crossing is allowed is called a phantom chain. We propose

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a phantom chain model for chromosome condensation and discuss its implications for the architecture of the eukaryotic chromosome. A preliminary account of this work has already been presented (Sikorav and Jannink, 1993b).

CHROMOSOME CONDENSATION: A SCHEMATIC DESCRIPTION

General features of the eukaryotic chromosome

Chromosomal DNA molecules, which can be several centimeters in length, are confined within the nucleus whose diameter is only a few micrometers. This necessitates the compaction (also called folding) of the DNA molecules. The compaction is achieved in a hierarchical manner and is regulated during the cell cycle. During the interphase, the degree of compaction is on average minimal; the maximal degree of compaction is found in the metaphase chromosome. Therefore the problem is schematically that of the change of compaction between the interphase and the metaphase chromosome.

There exist four basic levels of folding in the chromosomal chain (Alberts et al., 1990). First is the nucleosome, which consists of ~200 base pairs of DNA complexed with an octamer of histones. The nucleosome (rather than the base pair) is the elementary monomer in the chromosomal chain. A string of nucleosomes gives rise to the 11-nm filament. This filament is commonly described as having a more compact configuration in vivo, the 30-nm chromatin fiber, which is obtained by coiling of the 11-nm fiber into a solenoidal structure with about 6 nucleosomes per turn. The 30-nm chromatin fiber is apparently only present in the interphase chromosome as discussed below. The chromosomal chain is organized into domains (in the interphase) or loops (in the metaphase chromosome) containing from 30 to 100 kilobase pairs. At still a higher scale, there exists a macroscopic helical folding in the metaphase chromosome (Rattner and Lin, 1985; Boy de la Tour and Laemmli, 1988).

The description we give here is schematic for several reasons: The chromosomal DNA molecule is a heteropolymer. Therefore, strictly speaking, one cannot give a common description for two different chromosomes. Indeed, the heteropolymeric character of the DNA molecule is also reflected in the structure of the chromosome, where one can distinguish various types of segments (e g., eu- and heterochromatin, telomeres, centromeres, G and C bands, etc.; see Manuelidis, 1990, for a review). The structure of a given chromosome usually differs from one cell type to another in a given species (especially for the interphase chromosome) and is dynamically modified throughout the cell cycle. The distinction between only two extreme configurations (condensed and interphasic) is clearly a simplified description. The different experimental data available on the structure of eukaryotic chromosomes have been obtained in different species (yeast, *Drosophila*, etc.). It is not obvious that one may use the details of the data obtained from one species in the description of the chromosomes of other species.

In spite of these difficulties, it seems possible to give a schematic description of the two extreme states of the eukaryotic chromosome that is in general valid and allows physical reasoning. In the description given below, for the sake of simplicity, we do not mention the particular species and cell types from which experimental evidence has been obtained.

Structure of the metaphase chromosome

In this most condensed form of the chromosome, the chromosomal chain is folded into loops (30–100 kilobase pairs) arranged radially in the chromatid (Fig. 1 *A*). These loops were visualized by electron microscopy; upon removal of the histone proteins, the metaphase chromosome has a structure similar to a densely adsorbed polymer. The nonhistone proteins present at the bases of these histone-depleted loops constitute the scaffold. Topo II is the major protein of the scaffold. The metaphase chromosome contains two or three molecules of topo II per average sized loop.

The local structure of metaphase chromosomes was examined by cryo-electron microscopy after in situ vitrification, a technique which preserves their native structure (McDowall et al., 1986). According to this study, chromosomes have a homogeneous, grainy texture which on optical diffraction gives rise to a reflection corresponding to 11 nm. No superstructure or periodic order is discernible, although a partial alignment of the 11-nm filament is not ruled out. This has led to the conclusion that the basic local structure of the metaphase chromosome is that of a compact amorphous aggregation of the 11-nm filament. In particular, the structure of the 30-nm fiber is not preserved in this condensed form.

For our purpose, we conclude that the volume fraction of the nucleosomes in this condensed state is close (or equal) to 1, as in a melt of nucleosomes. We can also estimate the average DNA concentration in the metaphase chromosome: it is approximately equal to the concentration of a 200-base-pair fragment contained in the volume of one nucleosome (a cylinder about 11 nm in diameter and 5 nm in height). This gives an average DNA concentration of 460 mg/ml.

Structure of the interphase chromosome

Generally speaking, the structure of the interphase chromosome is more complex and less well known than that of the metaphase chromosome. The increase in complexity results in particular from the heterogeneity regarding condensation and from the interactions with the nuclear matrix. The features that appear essential to the problem under study are the following.

1. Individual interphase chromosomes are organized as discrete morphological entities (also called chromosome domains or territories), i.e., segregated rather than penetrating each other (Manuelidis, 1990; Haaf and Schmid, 1991).

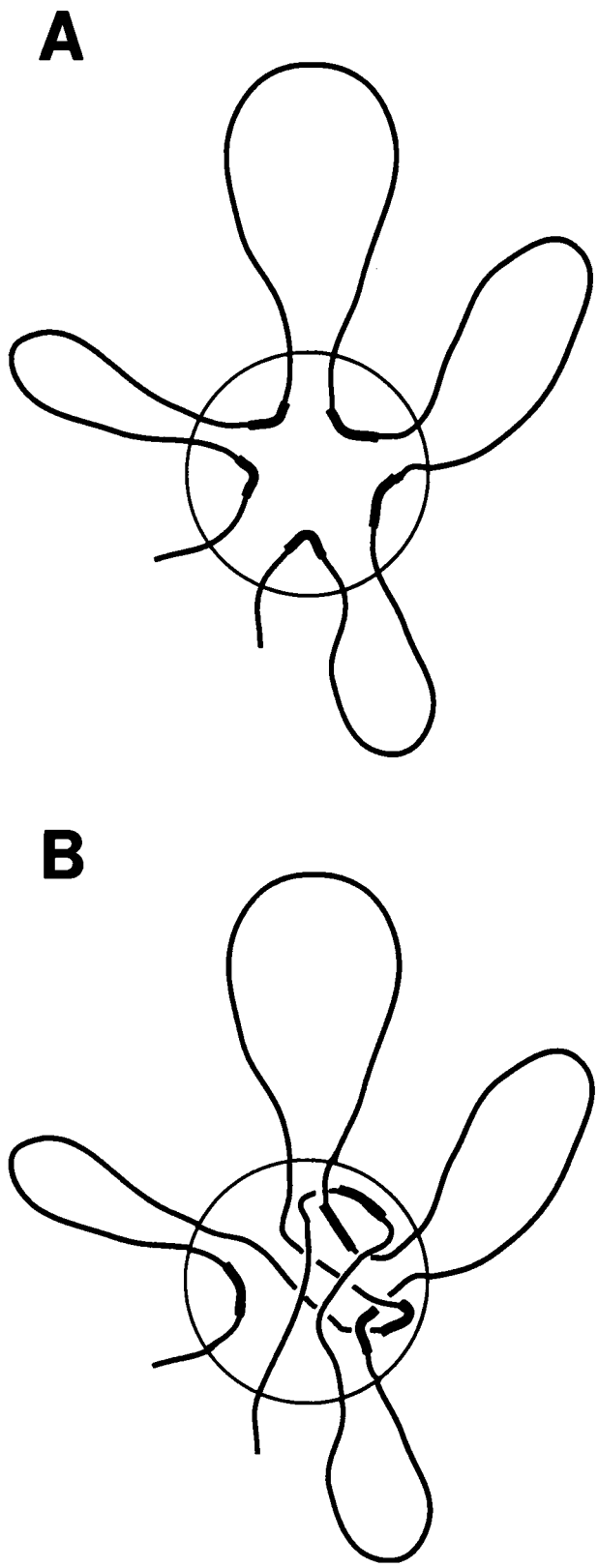

FIGURE 1 Schematic representation of the radial loop organization in a histone-depleted metaphase chromosome. DNA loops stem from the metaphase scaffold (*disk*). The scaffold-associated DNA sequences (SAR) are indicated by thick lines. The scaffold and the bases of the loops are shown enlarged (in comparison with the loops) to visualize the topological state of the chain. (*A*) Radial loop organization without self-knotting. (*B*) Radial loop organization with self-knotting.

2. Interphase chromosomes contain few or no self-knots. This assumption is supported by two lines of evidence: a) Radioautograms of interphase DNA usually show unknotted molecules (Sasaki and Norman, 1966; Kavenoff et al., 1974). Some circular molecules indicating self-knotting have been observed (Sasaki and Norman, 1966), but the size of the circles is not smaller than 1 cm, which corresponds to 30 megabase pairs of DNA. Therefore, by this criterion, the interphase chromosome contains less than one knot per 30 megabase pairs. b) On a smaller spatial scale (from 100 kilobases to 1–2 megabases), the structure of the DNA molecule observed by fluorescent in situ hybridization is Gaussian (van den Engh et al., 1992), which makes unlikely the existence of self-knots undetected by radioautography. We note here that the maximum size of these Gaussian portions (or blobs) is of the order of the radius of the chromosome territory (i.e., a few micrometers).

3. In contrast to the metaphase chromosome, the degree of condensation is not homogeneous within the chromatin. There exist more condensed regions (heterochromatin) and decondensed regions (euchromatin). The extent of decondensation varies from one cell type to another. In certain cases, the whole chromosome appears to be homogeneously decondensed. The average DNA concentration in the interphase chromosome ranges from 20 to 100 mg/ml, i.e., at least five times less than in the condensed chromosome. The local structure of the euchromatin is generally thought to be that of the 30-nm fiber.

4. The 30-nm fiber is organized in domains (50–100 kilobase pairs long) which are independent from the point of view of DNA supercoiling. These domains are generally thought to correspond to the loops present in the metaphase chromosome. The spatial organization of these domains in the interphase nucleus is still unclear. In the radial model, the interphase domains are represented as having preserved the metaphase loop structure (Fig. 1 *A*). However, this proposal seems difficult to reconcile with the investigations on the three-dimensional structure of the interphase DNA molecule using in situ fluorescent hybridization mentioned above (van den Engh et al., 1992). The fact that the DNA chain appears to be Gaussian for sequences separated by 100–2000 kilobase pairs does not seem to be compatible with the loop structure of Fig. 1 *A*.

Chromosome condensation: involvement of topo II

Before discussing the role of topo II in chromosome condensation, we recall the nature of the topological problems associated with double-stranded DNA, how they are dealt with by type I and type II topoisomerases, and the general role of these enzymes in mitosis.

There exist two types of topological constraints in double-stranded DNA (Watson and Crick, 1953, Frank-Kamenestkii and Vologodskii, 1981): the plectonemic constraint which arises from the intertwining of the complementary strands, and what can be called the "ordinary

polymeric constraint" which is present even if the plectonemic constraint is ignored.

Eukaryotic topoisomerases (either type I, which catalyzes the breakage and reunion of DNA strands one at a time, or type II) can remove positive and negative supercoils with equal efficiency. Thus, in principle, both types of enzyme should be able to deal with the problems associated with the plectonemic constraint.

Type II is strictly required at the time of mitosis. Topo I cannot substitute for topo II either in the condensation process or for the segregation of the sister chromatids in anaphase.

The requirement of topo II for segregation is reasonably well understood: the existence of multiple origins of replication leads to the intertwining of the sister chromatids; when the mitotic spindle pulls the sister chromatids, this intertwining becomes an obstacle similar to the obstacle created by the intertwining of replicated circular molecules. In the absence of nicks, only topo II can assist the decatenation of the two chromatids at anaphase (Sundin and Varshavsky, 1980; Wang, 1991).

In the case of the condensation process, the respective roles played by the two types of topological constraints is not understood. The possible role of supercoiling in the compaction of DNA molecules has been discussed by several authors (Boles et al., 1990). However, if the plectonemic constraint were the only topological constraint in the condensation process, topo I should be able to substitute for topo II (Wang, 1991). This leads us in this work to discuss the role of topo II in the condensation process focusing on the "ordinary polymeric constraint." It does not imply, however, that the plectonemic constraint plays no role in this process.

The participation of topo II in chromosome condensation has initially been established in vivo by genetic analysis in yeast and has also been investigated in detail in vitro (Laemmli et al., 1992). Chromosome condensation can be obtained in vitro by the addition of mitotic extracts to an interphase nucleus. In these experiments the interphase nucleus comes from terminally differentiated (G_0) cells: the DNA has not been replicated in contrast with the in vivo situation. After the dissolution of the nuclear membrane and the disassembly of the nuclear lamina, the process of chromosome condensation takes place in two stages. First, in the absence of topo II precondensed chromosomes accumulate. They consist of clusters of swollen chromatids which have a sausage-like shape. We shall see below that this is the form expected for the first stage of a coil to globule transition (de Gennes, 1985). Second, fully condensed mitotic chromosomes can be obtained by the addition of purified topo II. Dosage experiments show that a threshold concentration of topo II is required for complete condensation, which favors the idea of a structural involvement of topo II in the process. Such an involvement is further suggested by the fact that topo II can in vitro aggregate the specific AT-rich DNA sequences (scaffold-associated regions, SAR) to which it binds (Adachi et al., 1989). This supports the idea that topo II may act as a loop fastener, tethering the DNA loops to the scaffold through specific interactions with SAR sequences (Fig. 1 *A*).

However, topo II does not solely act as a condensing agent, as indicated by the facts that 1) other condensing agents such as histone H1 are unable to substitute to topo II; 2) inhibitors of topo II prevent full condensation; and 3) catalytically inactive topo II obtained by the substitution of a single amino acid cannot drive the condensation process, although it is still able to aggregate the SAR DNA sequences. Thus, the strict requirement for the catalytic properties of topo II is well established.

Summary

The process of chromosome condensation can be schematically described as the transition from a decondensed state (the interphase chromosome) to a condensed state (the metaphase chromosome). In the initial state, the chromosomal DNA chain is unknotted and segregated from the other chromosomal DNA chains. In the final state, the chain has a locally amorphous structure and possesses the density of a melt of nucleosomes. This density is at least five times larger than that of the interphase state. Topo II catalytic activity is strictly required to drive the condensation process. In addition, experimental evidences support the idea of a structural involvement of topo II in chromosome condensation.

KINETICS OF POLYMER RELAXATION

The onset of attractive forces causes the random coil to shrink into a globular state. We examine the conditions necessary to reach a given density of collapse and evaluate the characteristic times.

In the case of homogeneous polymers, the initial state of the process is necessarily the dilute solution state; at higher concentrations, the effect of attractive forces between monomers is to induce aggregation of chains, which remain in the random coil state. Copolymers react differently, and a globular melt is possible. In such systems, there are two distinct compensation temperatures, interchain and intrachain. Attractive forces can also exist between monomers and a surface. In this case, the polymer chain is adsorbed and its density near the surface can be as high as the polymer melt. The polymer may also be part of a macroscopic network, due to permanent or temporary bonds. In these constraining conditions, the shrinking is very conspicuous.

It is not obvious to select a process that provides an adequate model for a quantitative description of chromosome condensation. However, in this particular process, the density at the end of the collapse becomes as high as in the melt state. Thus, we are interested in the long relaxation times, and we expect to find similar behaviors in the last stages of all relaxation processes.

It has become useful to describe the dynamics associated with the tail of the relaxation time spectrum in terms of

entanglement effects. Such an expression will be used in this paper. The improvement with respect to the description related only to the relaxation spectrum is given by the positioning of the interactions leading to long relaxation times. This positioning is determined by the topology of the chain and by the fact that two sequences cannot cross each other. An entanglement is thus invoked at every place and time where two sequences attempt to cross each other. However, this concept is not yet fully elucidated, and experiments have been proposed for its clarification (Sikorav and Jannink, 1993a).

The model

We consider as a model the shrinking of polymer chains in dilute solutions, after a temperature drop, or equivalently, after the addition of a precipitant. This is the coil-globule transition (des Cloizeaux and Jannink, 1991). The use of this model raises two major problems: first, the fact that the process of chromosome condensation occurs in a nondilute solution, and second, that the density in the final state is the density of a melt. This density has usually not been observed in the collapse of neutral homopolymers, except recently in deep quench experiments (Yu et al., 1992). Regarding the first problem, we discuss below a possible mechanism (an intermolecular solubilization) that can explain the globulization of concentrated polymeric solutions. For the second problem, we note that in contrast to the loose globular structure commonly obtained with neutral polymers, dense globular states have been observed in the collapse of DNA molecules. For experimental evidence we give the results of Post and Zimm (1982), who have measured light scattering on double-stranded DNA in water plus ethanol plus added salt. They interpret their data as resulting from a mixture of dense spheres and aggregated random coils. The deswelling factor of the spheres is: $\alpha^2 = R^2/R_0^2 = 0.04$, where R is the radius of gyration of the sphere and R_0 the radius of gyration of the random coil. The final monomer volume fraction within the sphere is:

$$\varphi_f = \frac{1}{N^{1/2}\alpha^3}\,\varphi_{melt} \tag{1}$$

where N is the number of base pairs. For $N = 10^4$, $\varphi_f \approx \varphi_{melt}$, a result similar to that of Yu et al. (1992). Conditions can be determined to avoid the contamination of aggregated random coils: there are two types of conditions, steric and kinetics (Grosberg and Kuznetsov, 1993). The dilution requirement is such that no observation can practically be made in such a state. It seems, on the contrary, that the kinetics following deep quenches provides better results. It is, for instance, reported by Porschke (1984) that there are two characteristic times in the DNA condensation after the addition of spermine: the fast relaxation time (10^{-3} s) is associated with intramolecular condensation, whereas the slow relaxation time (10^2 s) concerns intermolecular aggregation.

Characteristic times in polymer dynamics

Our goal is to evaluate the time τ_{cg} necessary for a random coil to collapse into a dense globule. For this, we first consider known characteristic times in polymer dynamics that are related to the Brownian motion of random coils and examine to what extent τ_{cg} can be explicitly formulated in terms of these times.

The time τ_Z (or Zimm relaxation time) corresponds to the relaxation of a single chain from an extended state to a random walk state. Experimentally, it is derived from the measurement of viscosity in dilute solution. A calculation gives

$$\tau_Z \approx \eta_s R^2 R_h / k_b T, \tag{2}$$

where η_s is the solvent viscosity, R the radius of gyration, and R_h the hydrodynamic radius at equilibrium. We will see that τ_Z also describes the early stage of collapse, resulting in a crumpled state, which has the same topology as the initial state.

When free draining of the solvent is imposed, the corresponding relaxation time is τ_R, the Rouse relaxation time,

$$\tau_R \approx \zeta N R^2 / k_b T, \tag{3}$$

where ζ is the monomer friction. However, there is no direct measurement of τ_R.

In the later stage of the collapse, we need to account not only for free draining of the solvent but also friction between the monomers (internal viscosity) and topological constraints of entanglements. A significant relaxation time is the reptation time τ_{rep}, the time required for a Gaussian chain to travel by diffusion a distance R_0, in the presence of other chains. The variation of τ_{rep} with N, $\tau_{rep} \propto N^3$, reflects the presence of topological obstacles. Such a time is derived experimentally from self-diffusion measurements in melts. However, τ_{rep} varies strongly with the temperature difference $(T - T_g)$, where T_g is the glass transition temperature. This complication makes it difficult to apply τ_{rep} to describe the late stage of the collapse.

Kinetics at the onset of shrinking

Predictions have been given for the kinetics of chain collapse in the initial stages of the transformation. At time 0, the chain of N monomers has a random walk configuration. The number of self-contacts is then $N^{1/2}$. Assuming that N_e contacts are necessary to obtain an effective entanglement, we can write the condition for a dynamic phantom chain behavior as $N^{1/2} < N_e$.

In the melt state $N_e \approx 300$. If we use this number for the isolated random walk, we find a crossover for the onset of entanglement effects at $N \approx 10^5$. The kinetics of phantom chains has been described (de Gennes, 1985). In a first period, the contraction (to the 2.5 power) is linear in time, with a relaxation time proportional to N^2 (Rouse type of relaxation). In terms of measurable quantities, this

time reads

$$\tau_1(N) = \tau_Z(N) \frac{\Delta T}{\Delta T^*(N)} \quad (4)$$

where $\tau_Z(N)$ is the Zimm relaxation time associated with the Brownian motion of an ideal chain in the non-free-draining hypothesis and $\Delta T^*(N)$ is the difference between the Flory temperature T_F and the critical temperature $T_C(N)$ of phase separation between solvent and solute. The corresponding shrinking ratio is $\alpha^2(t) = (1 - t/\tau_1(N))^{1/5}$.

Eq. 4 is based on the existence of blobs, made of the greatest polymer sequences within which the configuration is Gaussian. The size of a blob is inversely proportional to ΔT. Sequences larger than blobs occupy space like segregated compact spheres.

At the end of the first period (called fast crumpling in Grosberg et al., 1988a), the anisotropic, compact state of blobs becomes spherical. Here the contraction is exponential (de Gennes, 1985). If we assume that the effective viscosity for the Brownian motion of a monomer is equal to the solvent viscosity η_s, the relaxation time is

$$\tau_2(N) = \tau_1(N)\left(\frac{g}{N}\right)^{3/5}. \quad (5)$$

An evaluation of the relaxation time for a non-phantom chain (i.e., with entanglement effects) is given following Grosberg et al. (1988a), where it is called the slow knotting phase.

A first stage is characterized by a relaxation time as in Eq. 4. It is, however, limited to a sequence of g^* monomers ($g^* < N$). g^* is the number of monomers necessary to create an effective entanglement in the kinetic process. The authors evaluate g^* as follows

$$g^* = N_e\, g \quad (6)$$

where g is the number of monomers in the Gaussian blob. It is thus assumed that there is a single contact between adjacent blobs and that N_e such contacts are necessary to build an effective entanglement. The relaxation process of Eq. 4 is thus valid within sequences of g^* monomers,

$$\tau_1(g^*) = \tau_Z(g^*) \frac{\Delta T}{\Delta T^*(g^*)}. \quad (7)$$

For larger sequences, we must include the relaxation of topological obstacles, as described in the following section.

Shrinking kinetics close to the melt state

As the compaction ratio α^2 becomes smaller than 1, the average number of self-contacts increases from $N^{1/2}$ to N. Simultaneously, the number of monomers per blob tends to 1. Clearly, $N \gg N_e$: the effects of entanglements are important. Several modifications must be introduced with respect to the initial stage. The effective local viscosity η_{ef} experienced by a monomer will be greater than the solvent viscosity η_s. We expect therefore an increase in the Rouse relaxation time, and we denote $\tau_{1ef}(N)$ the effective Rouse time in Eq. 4. Doi and Edwards (1986) propose (Eq. 5.190)

$$\tau_{1ef}(N) = \left(1 + \frac{\rho^2 b^6}{24} N\right)\tau_1(N), \quad (8)$$

where ρ is the monomer density and b the length of the monomer. In the compaction process, the chain statistics changes with the scale. For a sufficiently long sequence, $n > g$

$$r^2(n) \propto n^{2/3}. \quad (9)$$

For smaller sequences, $n \leq g$

$$r^2(n) \propto n. \quad (10)$$

This crossover generates a characteristic slowing down process in the kinetics of compaction, as noted by Grosberg et al. (1988a). However, the magnitude of the effect depends on the local stiffness of the chains. Let C be the (dimensionless) third virial coefficient. Grosberg et al. (1988a) expect an important effect if $N_eC < 1$. For polystyrene, $C = 3 \times 10^{-3}$, $N_e = 300$, and $N_eC \approx 1$.

Consider the deswelling of the polymer chain at fixed chain topology. This topology halts the relaxation process at scales greater than $g^* = N_e g$: the blobs are segregated and each blob is viewed by the others as an obstacle. To pass the obstacles, the chain behaves dynamically as branched polymers or closed loops. The statistics of such sequences is given by the relation $r^2(n) \propto n^{1/2}$, implying a hypercompact occupation of volume. The progression in such circumstances requires a time that is exponential in the sequence length n. Thus it becomes preferable for the total chain of length n to undergo a motion akin to reptation, in which the ends of the chain penetrate through the segregated blobs and thus remove the obstacles by a change of topology. The problem differs from the reptation process in polymeric melts because 1) the topological obstacles are created by the chain itself, and 2) the number of topological obstacles increases progressively during the compaction process. Grosberg et al. (1988a) propose as a crude evaluation for τ_{cg} the reptation time of a chain of blobs of g^* monomers

$$\tau_{cg}(N) \approx \tau_1(g^*)\left(\frac{N}{g^*}\right)^3. \quad (11)$$

Eq. 11 is based on relaxation in polymer melts for which the statistics are Gaussian.

The reference state is considered here to be the dense sphere. Thus $g = 1$ and $g^* = N_e$. We obtain

$$\tau_{cg}(N) \approx \tau_{1ef}(N_e)\left(\frac{N}{N_e}\right)^3 \quad (12)$$

where $\tau_{1ef}(N)$ is the modified Rouse relaxation time accounting for an effective viscosity in Eq. 8.

Summary

We have considered the coil-globule transition in dilute solution as a model for chromosome condensation. The model of Grosberg et al. (1988a) is based on the relaxation of topological constraints. It applies to the late stage of the compaction process. A slow reptation-like process carries the system into a more compact state. Provided that this is valid, the compaction times for very long chains of N monomers is estimated to scale as N^3.

A PHANTOM CHAIN MODEL FOR CHROMOSOME CONDENSATION

Kinetics of condensation in the absence of topo II

We treat the problem of chromosome condensation as the collapse of a homopolymer made of N nucleosomes from a decondensed state, the interphase chromosome, to a condensed state, the metaphase chromosome. The phenomenon is assumed to result from a decrease in the solvent quality within the cell at the time of mitosis. We have seen that the importance of the effects of topological obstacles depends on the local stiffness of the chains. In the case of the chromosomal chain of nucleosomes, the local stiffness is not known but, as described above, the late stage of the condensation process does not occur in the absence of topo II. Thus, the characteristic slowing down exists, implying that the local stiffness of the chain is such that $N_eC < 1$ (C being the third virial coefficient). Also, the initial stage is clearly in a more compact configuration than the random walk configuration. However, because the chain is unknotted and its density is much lower than the density of a melt of nucleosomes, we know that the condensation process requires a change in the topology of the chain and that the natural duration of this process (i.e., in the absence of topo II) is given by Eq. 12, which with Eqs. 7 and 8 yields

$$\tau_{cg}(N) \approx \left(1 + \frac{\rho^2 b^6}{24} N_e\right) \tau_Z(N_e) \frac{\Delta T}{\Delta T^*(N_e)} \left(\frac{N}{N_e}\right)^3 \quad (13)$$

or $\tau_{cg}(N) \approx aN^3$, where a is time, which we now estimate.

In the melt state, $\rho \approx v^{-1}$, where v is the volume of a monomer (a nucleosome): $v \approx 4\pi/3(b/2)^3$) with $b = 11$ nm. Thus, $[1 + (\rho^2 b^6/24)N_e] \approx 87$ (using $N_e \approx 300$).

We evaluate $\tau_Z(N_e)$ in the following manner: $\tau_Z(N_e) \approx \tau_{el} N_e^{3/2}$, where τ_{el} is the characteristic time associated with the motion of a monomer (i.e., a nucleosome). As a rough estimate for τ_{el}, we use the relaxation time associated with the rotation of a solid Brownian sphere of radius 5.5 nm. This gives a value of $\tau_{el} \approx 0.5 \times 10^{-6}$ s.

In addition, we have $\Delta T^*(N_e) \approx \Theta/N_e^{1/2}$ (from de Gennes, 1985), and since the final state has the density of the melt, the decrease in solvent quality must be described with an equivalent temperature quench $\Delta T \approx \Theta$. This gives

$$\tau_{cg} \approx 1.5 \times 10^{-7} N^3 \, s. \quad (14)$$

Comparing this estimate with the observed values (in the 10^2- to 10^4-s range), we conclude that the process cannot occur in the observed time, provided than N is greater than $\approx$870 (for an observed time of 10^2 s) or $\approx 4 \times 10^3$ (for an observed time of 10^4 s), which corresponds to a value of 1.7×10^5 to 8×10^5 base pairs. This result provides a tentative explanation of the strict requirement for the catalytic activity of topo II. We note that the whole interphase chromosome need not be decondensed for the reasoning to hold. Decondensed Gaussian blobs of about 1–2 megabase pairs are large enough to require the enzyme. For the largest known chromosomes (such as those of the lungfish, with an average size of about 5×10^9 base pairs), the rate acceleration brought about by the enzyme can exceed 10^{10}. This rate acceleration, although impressive, is not uncommon in the field of enzymatic catalysis (for example, see Jencks, 1975).

A phantom chain model for chromosome condensation

Assuming the value $N_e \approx 300$, we find that the average size of a loop (about 300 nucleosomes) is approximately equal to the mean distance between two entanglements. We correlate the number of topo II molecules (two or three per loop) in the metaphase chromosome with the number of entanglements. We tentatively propose that in the metaphase chromosome, each potential entanglement point is in fact dealt with by a topo II molecule. To understand the effects of topo II catalytic activity on the condensation dynamics, we consider the dynamics of the chromosomal chain near the end of the condensation process, when the volume fraction becomes close to the value of the melt state. In a crude approximation, we describe the mean square displacement of a monomer using the reptation model. We recall the equations describing the mean square displacement $\phi_n(t)$ of a monomer in this model (Eq. 6.112 in Doi and Edwards, 1986)

$$\phi_n(t) = b^2(t/\tau_{el})^{1/2} \qquad \tau_{el} < t < \tau_{Rouse}(\tau_{el}, N_e),$$
$$= b^2(t/\tau_{Rouse}(\tau_{el}, N_e))^{1/4}$$
$$\tau_{Rouse}(\tau_{el}, N_e) < t < \tau_{Rouse}(\tau_{el}, N),$$

where b is the length of the monomer and $\tau_{Rouse}(\tau_{el}, N) = \tau_{el}N^2$. Again, the reptation model requires Gaussian statistics.

It can be seen that the time $\tau_{Rouse}(\tau_{el}, N_e)$ denotes the onset of entanglement effects. For $t < \tau_{Rouse}(\tau_{el}, N_e)$, the chain behaves as a Rouse chain in free space; for $t > \tau_{Rouse}(\tau_{el}, N_e)$ the chain feels the presence of the obstacles. In our case, we have $\tau_{Rouse}(\tau_{el}, N_e) \approx 0.5 \times 10^{-6} \times (300)^2 s \approx 0.05$ s.

We apply these two equations to the description of the dynamics of the metaphase chromosome: in the presence of topo II, each entanglement point can be crossed with a time constant τ_{cross}. Since there is enough enzyme to saturate all the entanglement points, τ_{cross} is approximately equal to the

inverse of the turnover number of the enzyme (Sikorav and Jannink, 1993a). A plausible value for τ_{cross} is thus $\approx$0.1–1.0 s (see the discussion in Sikorav and Jannink, 1993a, and also Lindsley and Wang, 1993, for a recent determination of the turnover number). Thus τ_{cross} and $\tau_{Rouse}(\tau_{el}, N_e)$ are of the same order of magnitude. The presence of topo II leads to a suppression of the effects of the longer relaxation times. We call this phantom chain behavior. This does not mean, however, that the entanglements are not felt, but are simply less efficient.

There are two main consequences of the presence of topo II on the condensation process. One is on the kinetics of condensation. The slowdown in the condensation process due to the topological constraints is essentially abolished. The process becomes that of the collapse of a dynamic phantom chain and the time required is given by Eq. 4. Here, the size of the chain that must be taken into consideration is that of the maximum size of the Gaussian blobs present in the interphase chromosome (1–2 megabase pairs). Although these Gaussian blobs penetrate each other, we can ignore this fact because of the presence of topo II and consider the collapse of each blob as that of an isolated chain. This yields a time in the 10^2 s range, in agreement with the experimental values. This time scale is also compatible with the time scale on which topo II acts: in other words, the enzyme is effectively able to catalyze the release of the topological constraints during this period.

The other consequence of the presence of topo II on the condensation process is on the structure of the metaphase chromosome. As discussed above, chromosome condensation requires a change in the topology of the chain. Since this change cannot occur by the reptation-like mechanism, it must be driven by the catalytic activity of topo II. Taking into consideration the peculiar structure of the metaphase chromosome with its radial loop organization, the topological state of the condensed chromosome can be described schematically as shown in Fig. 1 *B*. Thus in the metaphase chromosome, the number of self-knots is of the order of the number of topo II molecules. The possibility that topo II could be involved in the self-knotting of the metaphase chromosome has been proposed previously (Liu et al., 1980). Fig. 1 *B* further indicates that a role of loop fastener for topo II is not necessarily required in the condensed chromosome: the loop structure could be maintained by the topological closure produced by topo II, as suggested earlier (Earnshaw and Heck, 1985; Laemmli et al., 1992). The recent observation that topo II can be extracted from mitotic chromosomes without disrupting the shape of the chromosome (Hirano and Mitchison, 1993) seems to be in agreement with this idea, favoring the model of Fig. 1 *B* rather than of Fig. 1 *A* (see also Swedlow et al., 1993).

DISCUSSION

We have previously hypothesized that under appropriate conditions the strand-passing activity of topo II could affect the dynamic properties of linear DNA molecules by releasing the topological constraints that give rise to the reptation motion (Sikorav and Jannink, 1993a). A theoretical analysis led us to conclude that this release could in principle be observed in a semidilute solution of long DNA molecules (more than approximately 1 megabase pair). This raises the question of whether the concepts of reptation and topological constraint release by topo II can be useful to the understanding of certain biological phenomena. Here we have studied the problem of chromosome condensation using a model proposed by Grosberg and co-workers (1988a, b) to describe the collapse of an isolated polymeric chain. It provides a tentative physical explanation for the requirement of topo II and allows the making of a verifiable prediction of the topological state of the condensed chromosomes. In the following section we discuss the validity of the model, the segregation of the chromosomal chains, and the appearance of the loops in the condensation process.

The model and its alternatives

There are important phenomena in the condensation process that are not accounted for in our model. The model proposed by Grosberg et al. (1988a) describes a collapse in which the final stage is an isotropic (spherical) globule. Metaphase chromosomes are not isotropic; in addition, they possess a macroscopic helical structure (Rattner and Lin, 1985; Boy de la Tour and Laemmli, 1988).

The fact that the interphase chromosome contains few or no self-knots is also very striking. According to Monte Carlo simulations (Frank-Kamenetskii and Vologdskii, 1981; Grosberg et al., 1988b), the probability P that a chain is not knotted as a function of the deswelling parameter α^2 is given by $P \approx \exp(1 - \alpha^{-2})$. If we suppose that a 30-megabase pair chromosome is made of about 30 1-megabase self-penetrating Gaussian portions, this corresponds to a deswelling parameter $\alpha^{-2} \approx 30$, which gives $P \approx 2.5 \times 10^{-13}$, in contradiction with the experimental observations of Sasaki and Norman (1966). This means that in the interphase chromosome there must exist some special mechanism that prevents self-knotting. This suggests the possible existence of a similar mechanism operating in the metaphase chromosome.

A process leading to an ordered structure, such as crystallization, would appear more plausible. Indeed, it has long been suggested that processes similar to the crystallization of polymers could provide a description of the folding of chromosomes (Rich, 1962). Furthermore, a liquid crystalline organization of the chromatin has been observed in many biological materials (Livolant, 1991), and chromosome condensation may occur in a non-uniform fashion, with particular chromosomal regions serving as foci in the process (Hiraoka et al., 1989).

All of these observations suggest a different picture of the condensation process, involving only helical coiling at each hierarchical level of folding (see, for instance, Manuelidis, 1990). In this picture, there is no need for chain knotting in the condensation process. The self-similar, amorphous

crumpled globule proposed by Grosberg et al. (1993a, b) also fits into this scheme. There are two difficulties with this scheme: in the metaphase chromosome there is practically no indication of a crystalline order according to Dubochet and co-workers (McDowall et al., 1986), and it does not explain the requirement of topo II.

To explain this requirement, a referee suggested that the failure of chromosomes to properly condense could be due to an inability to adequately segregate the interwound sister chromatids resulting from replication. We agree that this provides a plausible explanation for the in vivo observations. However, this reasoning cannot apply to the in vitro experiments in which unreplicated chromosomes were used.

A second aspect in which chromosome condensation could differ from the collapse described in Grosberg et al. (1988a) is the possibility of an adsorption on a surface. Certain regions of the chromosome could be adsorbed permanently or transiently to surfaces. Scaffold-associated regions, for instance, could become adsorbed to the metaphase scaffold, which would alter the description. We do not know how to treat this coupled transition, but we suspect that entanglement effects would again dominate the last stages of the process.

Thus, we are aware of the crudity of the model discussed in this work. It does not account for important aspects of either the condensation process itself or the final state of the metaphase chromosomes. However, the objections raised against this model do seem to invalidate it and do not offer an explanation for the requirement of topo II.

Finally, we note that the condensation process could be further slowed down by a glass transition. Indeed, physical gelation of DNA has been observed in concentrated solutions (Fried and Bloomfield, 1984). The reptation time given by Eq. 14 could constitute a lower bound rather than a true estimate for τ_{cg}. However, since the reptation time obtained from Eq. 14 is already too large to be compatible with the experimental time scale, the model of Grosberg and co-workers can still be used to explain the requirement for topo II.

Can we explain the segregation of the chromosomal chains?

A striking feature of the concentrated polymer solution made up by the different chromosomal chains is the segregation of the different chains observed throughout the cell cycle. There are, in fact, two distinct problems.

First, in the process of chromosome condensation, starting from segregated swollen chains, a decrease in solvent quality leads to the individual condensation of the different chromosomes without any aggregation. A plausible explanation for this phenomenon is provided by the concept of intermolecular solubilization of collapsed globules (Rička et al., 1991). This solubilization has been observed for a neutral polymer in an aqueous solution: in the absence of surfactants, the polymer aggregates; under the same experimental conditions, upon addition of a small amount of surfactant, the polymer can form a stable dispersion of isolated collapsed globules. In the case of the condensation process, it is known that the metaphase chromosome is almost entirely covered by a sheath which appears at early prophase and is present from prophase to telophase (Gautier et al., 1992). This sheath contains specific proteins such as perichromin, a protein localized in the nuclear envelope in the interphase (McKeon et al., 1984). To explain the lack of aggregation of the metaphase chromosomes, we propose that the sheath which surrounds them act as an intermolecular solubilizer.

The second problem concerns the reversal of condensation: the condensed chromosomes swell at the beginning of the interphase (from an amelioration of the solvent quality) and yet remain segregated. It is tempting to explain this observation by the decrease in the catalytic activity of topo II at the end of the cell division (Swedlow et al., 1993; Heck et al., 1988). The enzymatic activity would become too low to allow an interpenetration of the different chromosomes as phantom chains. In such a case, interpenetration requires reptation, and this process is too slow. However, at the same time, the enzymatic activity of topo II must be present to remove the knots of the condensed chromosomes. These two requirements appear to be contradictory.

Can we explain the appearance of the loops?

We have seen that experimental evidence does not seem compatible with the existence of the metaphase radial loop structure during the interphase. This raises the question of the mechanism of their appearance. Following Laemmli and coworkers (Laemmli et al., 1992; Adachi et al., 1989), the appearance of the loops results from the orderly aggregation of the scaffold-associated regions mediated by topo II. Thus, a structural role of topo II as a loop fastener would be required at the beginning of the condensation process but not in the fully condensed chromosome as discussed above. Laemmli's proposal appears to be supported by theoretical and experimental considerations: the description of the chromosomal chain is schematically that of an associating polymer, i.e., a flexible long chain molecule in a good solvent containing a small fraction of strongly associating monomers (or "stickers") (Baljon, 1993). The presence of such stickers in the chain can theoretically lead to a collapsed configuration of the chain (Baljon, 1993). Experimentally, the appearance of loops has been observed in bacteriophage λ DNA molecules (Ott et al., 1978). Heating in the presence of concentrated $Mg(ClO_4)_2$ leads to individual condensation of the molecules into radially symmetrical "rosettes" composed of multiple loops. Furthermore, the bases of the loops appear to be made with the AT-rich regions of the λ DNA. The similarity to the radial loop structure present in the metaphase chromosome is striking and provides a strong evolutionary argument in favor of the loop model.

In the path to condensation described here, which presumably is the path followed in vivo, the first step is mediated by the binding of topo II to the scaffold-associated regions, in otherwise "good solvent" conditions. We note, however,

that another path is possible, at least in vitro (Laemmli et al., 1992), in which the first step is the decrease in solvent quality, followed by the addition of topo II. Although this alternate path also leads to a fully condensed chromosome, it is possible that the local (loop) structure of the condensed chromosomes obtained by the two pathways differs.

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