The Mitotic Chromosome Is an Assembly of Rigid Elastic Axes Organized by Structural Maintenance of Chromosomes (SMC) Proteins and Surrounded by a Soft Chromatin Envelope*

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The structure of mitotic chromosomes is still poorly understood. Here we describe the use of a novel approach based on elasticity measurements of a single chromosome for studying the organization of these objects. The data reveal that mitotic chromosomes exhibit a non-homogenous structure consisting of rigid elastic axes surrounded by a soft chromatin envelope. The chemical continuity of DNA, but not RNA, was required for the maintenance of these axes. The axes show a modular structure, and the structural maintenance of chromosomes (SMC) proteins participate in their organization. Topoisomerase II was not involved in either the organization of the axes or the maintenance of the mitotic chromosomes. A model for the assembly and the structure of the mitotic chromosome is proposed. According this model, the chromosome axes are dynamic structures that assemble at the onset and disassemble the end of mitosis, respectively. The SMC proteins, in addition to maintaining axis elasticity, are essential for the determination of the rod-like chromosome shape. The extreme compaction of mitotic chromosomes is determined mainly by the high amount of bivalent ions bound to DNA at mitosis.

DNA is highly compacted ~ 10,000–20,000 times in the mitotic chromosomes of a typical vertebrate cell. It is far from clear how this folding is accomplished. The 30-nm chromatin fiber accounts only for 40:1 of the compaction ratio, and the remaining ~ 500-fold compaction is achieved through a largely unknown mechanism (1, 2). The complexity of the mitotic chromosome structure has both fascinated and frustrated scientists for many decades. Structural analysis of mitotic chromosomes has been very difficult because of the extreme compaction of the chromatin fibers. A number of models for the chromosome structure have been proposed, ranging from a hierarchical folding of chromatin (3) to spaghetti-like disorder (4). The favored textbook model describes the structure of the mitotic chromosome as an assembly of chromatin loop domains attached to a central protein scaffold (5). This scaffold showed the same shape as the mitotic chromosomes, and this shape was preserved even upon removal of >95% of the chromosomal proteins and 99% of the DNA and RNA (5, 6).

Topoisomerase II and SeII, a structural maintenance of chromosomes (SMC)1 protein, were identified as the major components of the scaffold. About 70% of the total amount of topoisomerase II was found in the mitotic chromosome scaffold fraction (7). Several reports have suggested that this enzyme might occupy discrete foci that could correspond to the base of the chromatin loop domains (7, 8). It was proposed that topoisomerase II, in addition to its important enzymatic function in chromosome assembly, was a part of the structural framework of the mitotic chromosome (7, 8).

The SMC proteins exist in the cell as high molecular mass complexes, one class of which is termed condensins (9). The biochemical manipulations of extracts isolated from Xenopus eggs have identified two forms of condensins, 8 S and 13 S. The active form of condensin, 13 S condensin, consists of two SMC subunits (XCAP-C and -E) and three non-SMC subunits (XCAP-D2, -G, and -H). Depletion and rescue experiments in Xenopus egg extracts have demonstrated that 13 S condensin is required for both assembly and maintenance of mitotic chromosomes (9).

The models reported in the literature are derived mainly from experiments using microscopy techniques. Recently, a novel and complementary approach via monitoring the changes in the elastic response was applied to study the organization of mitotic chromosomes (10–13). The elasticity is determined by the underlying structure of the studied materials and reflects the interactions holding them together. The knowledge of the elasticity properties of the studied object allowed its structure to be properly modeled. For example, the measurements of the bending rigidity and the force-extension curve showed that chromosomes assembled in Xenopus egg extract display a strong anomaly in their elastic response, i.e. their flexibility was found to be 2000 times higher than what could be expected from the measurement of their longitudinal deformability (11). This strongly suggests the presence of thin rigid axes inside these chromosomes, the diameter of which can be estimated, from elasticity calculation, to be ~20 nm (11). The axes are surrounded by a soft chromatin envelope (11). However, no direct data for such organization are yet available.

In this work, we report a detailed study on the structural organization of mitotic chromosomes assembled in Xenopus egg

* This work was supported by CNRS and INSERM. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ Supported by La Ligue contre le Cancer and the Association pour la Recherche sur le Cancer (ARC).

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1 The abbreviations used are: SMC, structural maintenance of chromosomes; pN, piconewton(s).
extract by using new approaches for investigating their elasticity. It is shown that chemical continuity of chromosomal DNA is essential for the maintenance of mitotic chromosomes. Direct experimental evidence is presented showing that the structure of the mitotic chromosome is heterogeneous and consists of a soft chromatin envelope and rigid elastic axes. SMC proteins, but not topoisomerase II, are involved in the organization of the mitotic chromosome. Interestingly, topoisomerase II was found to contribute to the stiffness of the whole chromosome. The data are summarized in a model of the mitotic chromosome that explains their main structural and functional properties.

EXPERIMENTAL PROCEDURES

Preparation of Mitotic Extracts—Xenopus egg extracts were prepared mainly by using the protocol of Losada et al. (14). Briefly, after removal of the jelly by cytoine treatment, the eggs were resuspended in XBE2 buffer (100 mM KCl, 2 mM MgCl2, 0.1 mM CaCl2, 10 mM potassium HEPES, pH 7.7, 5 mM potassium EGTA, and 0.05 M sucrose) supplemented with protease inhibitors (10 μg/ml of leupeptin and aprotinin) and 100 μg/ml of cytochalasin D. The eggs were crushed by centrifugation at 16°C (20 min at 15,000 rpm in an SW41 rotor; Beckman Instruments). The cytoplasmatic fraction was collected by puncturing the side of the tube with a 1-ml syringe, and leupeptin, aprotinin, cytochalasin D (final concentration of 10 μg/ml), and one-twentieth volume of 20× energy mix (20 mM phosphocreatine, 2 mM ATP, and 5 μl/ml kinase final concentration; final concentration) were added to it. Then the crude extract was transferred to 2-ml tubes used for a TLS-55 rotor (Beckman Instruments) and spun at 52,000 rpm for 2 h at 4°C. The lipid layer was removed carefully with a help of a vacuum, and the golden layer fraction was recentrifuged under the same conditions for 45 min. The supernatant was collected, aliquoted in 25-μl fractions, and frozen in liquid nitrogen. The extract was stored at −80°C.

Isolation of Demembranated Xenopus Sperm Nuclei and Chromosome Assembly—Xenopus demembranated sperm nuclei were prepared as described (15). The demembranated sperm was stored at −80°C and thawed immediately before use. The assembly of mitotic chromosomes was performed according to the described protocol (16). After completion of the chromosome assembly reaction, 5 μl of the reaction was transferred to the chromosome-stretching chamber, which contained 300 μl of EB solution and 150 mM NaCl, and the stretching was carried out. To test if the increase of the ionic strength removes topoisomerase II, a chromosome assembly reaction was brought to 100–200 mM NaCl, and the chromosomes were pelleted by centrifugation on a bench-top centrifuge. The pellet was washed with EB containing NaCl at 100–200 mM concentration and used for immunoblotting. The immunoblotting was carried out following a previously described procedure (16) and using an immunopurified anti-Xenopus topoisomerase II antibody (17).

Enzymic/Micromechanical Experiments—To study the role of nucleic acids or proteins in the maintenance of the mitotic chromosome structure, a series of enzymatic and micromechanical experiments was carried out. A typical experiment was performed as follows. A single chromosome was caught by the two pipettes through aspiration, and a small tension was applied. Then a third micropipette, which contained the enzyme, was moved near the chromosome, and the enzyme solution was sprayed onto it while maintaining the tension. Phase contrast and fluorescence images were acquired through a CCD camera and recorded on a VCR. In some experiments the elastic response of both the intact (before injection of the enzyme) and the digested chromosome was measured.

Four different enzymes, namely DNase I (10 units/μl; Roche Molecular Biochemicals), RNase A (500 μg/ml; Sigma), proteinase K (1 mg/ml; Sigma), and trypsin (50 μg/ml; Sigma) were used for spraying. It should be noted that the actual concentration of the sprayed enzyme on the chromosome was decreased by a factor of roughly 2–3 in comparison with that within the pipette as judged by experiments with fluorescent dyes (data not shown). When the spraying onto the chromosome was stopped, the enzymatic reaction arrests immediately due to the diffusion-mediated dissipation of the enzyme. In the case of the spraying of trypsin, the changes in the morphology and the elastic response of the chromosome due to the trypsin digestion were correlated with the SMC protein cleavage. Because it was not possible to visualize the degree of protein cleavage on a single chromosome, the digestion of a multitude of chromosomes by trypsin in conditions similar to those for a single chromosome was studied. Briefly, a chromosome assembly reaction was appropriately diluted with EB (see the section above titled “Chromosome Micromanipulations”), trypsin was added at concentration of 50 μg/ml, and the digestion was allowed to proceed for the appropriate time at room temperature. The chromosomes were then pelleted for 30 s on a bench top centrifuge, and the pellet was washed very quickly with ice-cold EB and recentrifuged for 15 s. After removal of the EB solution, the trypsin-digested chromosomes were immediately resuspended in SDS electrophoresis loading buffer and heated at 96°C for 5 min. The chromosomal proteins were separated on an 8% polyacrylamide gel containing SDS. The immunoblotting was carried out by using immunopurified anti-SMC protein (anti-XCAP-E; Ref. 9) antibody. It should be noted that, in the different trypsin digestion experiments, a complete cleavage of the SMC proteins was observed 2–4 min after the onset of digestion reaction.

RESULTS

The Chemical Continuity of DNA Is Crucial for the Maintenance of Mitotic Chromosome Structure—Elasticity studies have shown that native (10) or in vitro assembled (11) chromosomes could be elongated by close to two orders of magnitude of their initial length without breaking. This demonstrates that a mitotic chromosome contains a very large reservoir of length, their initial length without breaking. This demonstrates that a mitotic chromosome contains a very large reservoir of length, which is released gradually as a function of the applied tension. It is difficult to imagine that this peculiar property could be determined by the chromosomal proteins only. Thus, DNA and some nucleoprotein structures should be involved in these specific properties of mitotic chromosomes. If this is correct, one could expect the continuity of DNA to be essential for the maintenance of the elastic properties of the chromosomes. Hence, cleavage of chromatin DNA by nucleases would result in perturbation of the overall structure of the mitotic chromosome and loss of its stability. Moreover, the application of a small tension on the cleaved chromosome should lead to its dissolution.

To test this hypothesis, a single in vitro assembled chromosome was digested with deoxyribonuclease I, and the morpho-
logical changes resulting from the enzyme cutting of chromosomal DNA were observed. The experimental approach used is described on Fig. 1A. Briefly, demembranated Xenopus sperm nuclei were incubated in extracts isolated from Xenopus eggs. After ~150 min of incubation, well separated mitotic chromosomes were formed (Fig. 1B). Because these chromosomes are free in the extract solution, they can be easily manipulated. Initially, one end of the chromosome was aspirated and fixed to a pipette. Then, the other chromosome end was fixed to another pipette also by aspiration, and some small tension was applied to the chromosome (Fig. 1C). Next, a solution of DNase I was injected through a third pipette, and the structural alterations of the chromosome were visualized by both phase contrast and fluorescence microscopy (Fig. 1C). After some initial decrease of the overall diameter, the chromosome breaks into two halves (Fig. 1C). When a buffer solution alone was injected, no changes in the chromosome structure were observed (not shown, but see also Fig. 1E). This demonstrates that the chromosome integrity depends on the intactness of DNA.

Chromosomal Proteins, but Not RNA, Participate in the Maintenance of Mitotic Chromosome Structure—It is accepted that the chromosomal proteins are important players in maintaining the mitotic chromosome structure. To investigate the degree of protein contribution in the maintenance of the structural integrity of mitotic chromosomes, an experiment similar to the one described above was carried out, substituting proteinase K for DNase I (Fig. 1D). After some initial decondensation, the chromosome “melts” and, after 25 s of digestion, completely disintegrates. The melted chromosome did not exhibit any detectable elastic response (not shown). Thus, as expected, the presence of chromosomal proteins is crucial for chromosome structural organization.

Recent data have suggested a role for RNA in the maintenance of condensed heterochromatin (18). In addition, the high molecular mass Xist RNA is involved in the maintenance of the highly compact inactive X chromosome (19). This suggests a function for RNA in stabilizing the structure of interphase chromosomes. To test if RNA plays some role in the organiza-
Mitotic Chromosome Structure

Mitotic Chromosomes Exhibit a Non-homogenous Structure—
The above data demonstrate that both the DNA and chromosomal proteins are key factors in chromosome organization and structure. The chromosomal proteins and DNA might be assembled into a chromosome in different ways, thus forming either a non-homogenous or a homogenous structure. Most of the available models suggest that the chromosome exhibited a non-homogenous structure. However, recent elasticity experiments have modeled the chromosomes isolated from newt as solid, elastic rods (13). These data indicate an essentially homogenous structure formed of few rigid axes surrounded by a soft envelope of chromatin (11). Hence, a controversy exists in the literature. To clarify this controversy, we developed a new antibody-based micropipette technique to stretch a single chromosome and investigate its elasticity. The classical approach uses aspiration to fix both ends of the chromosome to two micropipettes with different rigidity (Fig. 2A; see also Ref. 10). Once the chromosomal ends are fixed, the chromosome is stretched by moving one of the pipettes (Fig. 2B). The aspiration technique allows us to study the elastic properties of the whole chromosome. From the force-relative extension dependence, the stretch modulus (the force necessary to elongate the chromosome twice its initial length, i.e. the slope of the force-relative extension curve) can be measured. The stretch modulus is a characteristic of the underlying structure of the object. A high stretch modulus reflects the fact that a high force is needed to elongate the object.

The new technique uses antibody-coated pipettes, and the “catching” of the chromosome is carried out through an antibody (Fig. 2A). Because an antibody against specific proteins can be used, this technique allows us to study selectively the elastic properties of mitotic chromosome domains that are associated with these proteins. If the chromosome structure is non-homogenous, one might expect different elastic responses when different antibodies (raised against proteins associated with different chromosomal domains) are used. In addition, the elastic response of the whole chromosome (when caught by aspiration) might also differ from the elastic responses of the different domains.

To study the elastic response of chromatin, we used highly specific anti-histone antibodies that allowed us to fix the chromatin fibers to the pipettes. A typical force-extension dependence is shown on Fig. 3A. As shown, the slope (the stretch modulus) of this curve is several times smaller than the slope of the force extension curve for the whole chromosome that is obtained when aspiration is used to fix the chromosomal ends to the pipettes (Fig. 3A). Histograms, presenting a summary of the stretching of 18 chromosomes with anti-histone antibodies and 11 chromosomes through aspiration, are presented on Fig. 3, B and C. The data show a peak of the stretch modulus of ~30 pN when chromosome stretching was performed through the anti-histone antibodies (Fig. 3C). However, stretching through aspiration gave an average of the stretch modulus of ~120 pN.
Hence, higher forces are necessary to elongate the chromosome when it is fixed through aspiration to the micropipettes compared with the forces needed to stretch the chromosome when the fixation is carried out through the chromatin fibers. Therefore, the chromosomes exhibit a non-homogenous organization with a lower elastic response for the chromatin entity, i.e. mitotic chromatin shows a relatively soft structure.

Bearing this in mind, one should expect the existence of chromosomal structures that are more rigid and show higher elastic response. The available data suggest that topoisomerase II and, more particularly, the SMC family of proteins might be associated with such structures (5, 7, 8, 21, 22).

The SMC Proteins Are Associated with Chromosome Regions Exhibiting Higher Elastic Response—To study the elastic properties of chromosomal regions associated with the SMC proteins, we initially performed the following experiment. A single mitotic chromosome was fixed by aspiration between two micropipettes, and the chromosome was elongated and then retracted to measure its native elastic response. A small tension was then applied to the chromosome ends, and a solution of the relatively weak protease (in comparison to proteinase K) trypsin was injected on the chromosome through a third pipette (see Fig. 1 for details). After 15 min, the injection of trypsin was stopped, the third pipette removed, and the chromosome was stretched again to measure its elastic response (Fig. 4). In contrast to proteinase K or DNase I treatment, digestion with trypsin did not strongly affect the overall morphology of the chromosome (Fig. 4, inset 2). Indeed, after 15 min of digestion with trypsin, both the diameter and the length of the chromosome increased 2-fold, but its elongated shape was preserved, and no chromosome breakage was observed. Trypsin digestion, however, has a pronounced effect on the elasticity of the chromosome; the stretch modulus of the trypsin-digested chromosome decreased from 3 to 4 times in different experiments (Fig. 4). This strongly suggests that trypsin has cleaved some proteins that play an essential role in the elastic properties of the chromosome. Because the SMC proteins were one of the primary candidates for such a role, we concentrated on them. We asked if the treatment with trypsin resulted in cleavage of these proteins. Because it was impossible to carry out such experiment on a single chromosome, we carried it out with an

Fig. 3. Elasticity measurements reveal a non-homogenous structure of mitotic chromosomes. A, two typical force-to-deformation curves for individual mitotic chromosomes obtained through aspiration (circles) and anti-histone antibody adhesion (stars). \( \Delta L/\Delta L \) is the elongation induced by the force. Note the large difference in the slope (the stretch modulus) of both curves. B, a histogram summarizing the measurements of the stretch modulus of 11 individual chromosomes by the aspiration technique. The ordinate designates the number of chromosomes studied, whereas on the abscissa are noted the measured stretch modulus. C, same as panel B, but for the measured stretch modulus of 18 individual chromosomes by the anti-histone antibody adhesion technique.

Fig. 4. Cleavage of SMC proteins with trypsin correlates with a decrease of the chromosome elastic response. A chromosome was suspended between two micropipettes and subjected to an extension-retraction cycle to measure its elastic response. Then the chromosome was relaxed, a slight tension was applied to both of its ends, and trypsin at a concentration of 50 \( \mu \)g/ml was injected by a third micropipette as described in the legend. After 15 min, the trypsin injection was stopped, and the chromosome was stretched again (curve labeled Tryp). The curve labeled Ctrl shows the elastic response of the same chromosome before digestion with trypsin. \( \Delta L/\Delta L \) is the elongation induced by the force. On inset 1 is shown the Western blot using anti-SMC antibody of non-digested and trypsin-digested (50 \( \mu \)g/ml for 5 and 10 min) mitotic chromosomes (for details, see Experimental Procedures’). Inset 2 shows the changes in the chromosome structure after digestion with trypsin for 5 and 10 min, respectively. Fluorescence images only are presented.
ensemble of mitotic chromosomes that were digested with the protease under conditions similar to those of the trypsin injection experiments (for details, see “Experimental Procedures”). The Western blot using anti-SMC antibody (anti-XCAP-E) of the trypsin-digested chromosomes clearly shows that the SMC proteins were proteolized (Fig. 4, inset 1). Therefore, the cleavage of the SMC proteins correlated with the reduction of the elastic response. This is indicative of a role for this family of proteins in maintaining the elasticity of mitotic chromosomes. Because, however, the treatment with trypsin affects the integrity of numerous proteins (data not shown), the decrease in the elastic response of the trypsinized chromosomes could not be attributed solely to the cleavage of the SMC proteins.

To clarify the function of the SMC proteins in the organization of the mitotic chromosomes, we have carried out stretching experiments by using anti-SMC antibody-coated (anti-XCAP-E antibody) pipettes. A typical force-extension curve with the anti-SMC antibody-coated pipettes is presented on Fig. 5A, curve 1. For comparison, a force-extension curve measured with the anti-histone antibody-coated pipettes is also shown (Fig. 5A, curve 2). Fig. 5B summarizes the results of the stretching of 22 individual chromosomes using the anti-SMC antibody. The stretching modulus exhibits a very large distribution with a mean value of \(-180–200\) pN. Note that the values of the stretch moduli of the SMC-associated regions are much larger than those of the chromatin envelope, and some of them are even larger than those of the chromosome when caught by aspiration. These results imply that the SMC proteins are associated with chromosomal regions with higher elasticity response.

**Topoisomerase II Is Not Involved in the Maintenance of the Mitotic Chromosomes**—To study the involvement of topoisomerase II in the maintenance of the in vitro assembled mitotic chromosomes, we used a peculiar property of these chromosomes, i.e. the possibility to remove topoisomerase II by increasing the ionic strength of the solution (17). Indeed, in agreement with Ref. 17, increasing the NaCl concentration of the solution to 100 mM led to removal of topoisomerase II (see the inset in Fig. 6A) but not of SMC proteins (data not shown) from the chromosomes. Treatment of the chromosomes with 100–150 mM NaCl slightly affected the overall shape of the chromosomes (data not shown, but see Ref. 17). The ends of a chromosome in a solution of 150 mM NaCl were fixed by aspiration to the pipettes, and a force-extension cycle was carried out. The force-extension cycle of a chromosome in 150 mM NaCl was identical to that obtained in the absence of NaCl (data not shown).
shown). The stretch modulus values of seven individual chromosomes measured in a solution of 150 mM NaCl are presented on Fig. 6A. As seen, the values of the chromosome stretch moduli in 150 mM NaCl fall essentially in the same interval of forces as those of chromosomes in the absence of NaCl. Therefore, the elastic response of the mitotic chromosomes is not affected by the absence of topoisomerase II. Because the elastic response reflects the underlying structure of the studied object, one can conclude that topoisomerase II does not participate in the maintenance of the structure of mitotic chromosomes.

To further study the structural role of topoisomerase II, we have carried out a series of chromosome-stretching experiments by using anti-topoisomerase II antibody-coated pipettes. Fig. 6B shows the summary of the data. The measured stretch moduli were similar to the stretch modulus obtained with anti-histone antibody-coated pipettes (compare Fig. 6B with Fig. 3C). Hence, topoisomerase II is associated with regions showing elastic response close to that of the soft chromatin envelope.

**DISCUSSION**

In this work, extracts from Xenopus eggs were used to assemble mitotic chromosomes. Because these chromosomes are easy to manipulate, this has allowed their inner organization to be studied via novel physical approaches for measuring the elasticity of a single chromosome.

The Elasticity Measurements Demonstrate a Heterogeneous Structure of the Mitotic Chromosomes—The use of either aspiration or anti-histone antibody-coated pipettes, two different ways to catch the ends of a single chromosome and study its elastic response, has demonstrated that the mitotic chromosome exhibits a heterogeneous structure. Indeed, when the ends of the chromosome were grabbed by aspiration, the average value of the stretch modulus was ~120 pN, whereas when anti-histone antibody coated pipettes were used this value was ~30 pN. The elastic response of chromatin was therefore much lower than that of the whole chromosome. This suggested that the chromosome should possess some regions with higher elastic responses. By using anti-SMC antibody-coated pipettes, the existence of such regions was demonstrated; the elasticity of the SMC-containing chromosomal regions was found to be at least as high as that of the whole chromosome. The data are compatible with a structure of the mitotic chromosome consisting of rigid elastic cores surrounded by a soft chromatin envelope (see the model presented on Fig. 7B).

The results clearly show that the SMC proteins are involved in the maintenance of the elasticity of the cores because: (i) cleavage of these proteins by trypsin correlates with a reduction in the chromosome elasticity; and (ii) the SMC-associated chromosomal regions are more resistant to elongation. Topoisomerase II, however, does not participate in the structural organization of the cores. Indeed, chromosomes depleted of topoisomerase II by NaCl treatment showed the same elastic response as the non-NaCl-treated ones, and the elastic properties of the chromosomal domains containing topoisomerase II were very similar to those of the soft chromatin envelope. Interestingly, DNase I digestion demonstrated that the chemical continuity of DNA is crucial for the elastic properties of the axes and the integrity of the mitotic chromosome in general. These results are in complete agreement with the recent data of Poirier and Marko, who showed that an individual newt chromosome was dissolved upon digestion with micrococcal nuclease or restriction enzymes (20). Therefore, the chromosome axes exhibit a specific chromatin structure, where the SMC proteins are main actors.

Organization of the Chromosome Axes—How are the axes organized? The analysis of the force-extension dependence of a single chromosome at high deformation could shed light on this question. As we have shown in the past, an irreversible transition in the structure of the whole chromosome (caught by aspiration) was observed at high deformation (11). Upon successive deformation cycles with a gradual increase of the extension, the chromosome was softened and, for each subsequent cycle, it was necessary to use a lower force to reach the same extension (see Fig. 5 in Ref. 11). The same type of elastic behavior of the chromosome at high deformation was observed when anti-SMC antibody-coated pipettes were used (data not shown). This is not surprising, because the elasticity of the whole chromosome is determined mainly by the elasticity of the rigid axes. The above type of elastic behavior is well described by a modular type of structure of the axes (11). Because the SMC proteins are associated with the axes, one could imagine that a single module of the axis contains one or several SMC (condensin) protein complexes. Given that the two coiled-coiled domains of the SMC proteins are organized around a structurally flexible hinge (23), the elastic response of the chromosome could reflect at least partially the elasticity of the hinge.

What is the role of the axes in the mitotic chromosome structure? We suggest that the axes, in addition to maintaining the elasticity of mitotic chromosomes, might be essential for determining the rod-like shape of mitotic chromosomes. Because the SMC proteins are associated with the chromosome axes, one should expect them to be involved in this event. If this is correct, a perturbation of the interaction of condensins with the axes would affect the shape of the chromosomes. In fact, this is the case; when anti-SMC antibody (an antibody against an Xenopus SMC protein, XCAP-C) was added to in vitro assembled mitotic chromosomes, the chromosomes lost their rod-
like shape and were converted into a shapeless chromatin mass (data not shown, but see also Fig. 5 of Ref. 24). Interestingly, the volume of this shapeless chromatin mass seems to be no more than 3–4 times greater than the one of the mitotic chromosome. This result is in complete agreement with the trypsin digestion data of a single chromosome presented on Fig. 4. Indeed, upon trypsin treatment for 10 min, the increase of the chromosome volume is not greater than 5–6 times that of the chromosome before the injection of trypsin (Fig. 4, inset 2, and data not shown). The elongated shape of the digested chromosome was preserved due to the small tension applied to both of its ends. Upon trypsin digestion, however, many other chromosomal proteins were cleaved in addition to the SMC proteins (data not shown). Thus, the above-described increase of the volume of the trypticized chromosome should also reflect the contribution of these proteins in maintaining the compact state of the mitotic chromosome. Hence, the presence of the SMC proteins would not affect significantly the overall compaction of the mitotic chromosomes.

Mechanism of Mitotic Chromosome Assembly—How do the chromosomes assemble during mitosis? Based on the above, the condensation process should necessitate the presence of the axes. One should also bear in mind that about half of the DNA phosphate groups are not neutralized in interphase chromatin (25) and that the association of bivalent and polyleptons with chromatin results in its higher compaction (25, 26). In addition, a recent ion microscopy study reported that the bivalent ions Mg$^{2+}$ and Ca$^{2+}$ are implicated in higher order mitotic chromosome structure through electrostatic neutralization (27). Indeed, in mitosis the amounts of Mg$^{2+}$ and Ca$^{2+}$ bound to chromosomes were found to be drastically increased compared with those bound to interphase chromatin (27).

Because the majority of condensins are not active in interphase, the axes would be formed during G$_2$/M in a process requiring active condensin complexes associated with the chromosome (Fig. 7A). This process would lead to some condensation of the chromosome, which would consist mainly in spatial chromatin fiber reorganization without substantial compaction of the chromatin. As a result, the chromosome would adopt an elongated shape. The higher chromatin compaction would be dictated rather by the higher amount of the bivalent ions Mg$^{2+}$ and Ca$^{2+}$, which bind to chromatin at mitosis (27). In agreement with this, upon antibody inactivation of the condensin complexes (24) or cleavage of SMC proteins with trypsin (this work), a relatively modest effect on the overall compaction of the chromosome is detected. In addition, removal of bivalent ions leads to impressive chromosome decondensation (27).

Thus, the condensin complexes could be viewed as organizers of correct chromosome condensation, whereas the chromatin compaction would, to a greater extent, be carried out by the increased amounts of bivalent cations bound to mitotic chromatin (Fig. 7A). Such a scenario seems reasonable, because it is not otherwise easy to explain how the limited number of condensin complexes alone (one active 13 S condensin complex per 10–20 kb of DNA) could be able to condense chromatin (28).

Our data are summarized in a model of the structure of mitotic chromosome presented in Fig. 7B. The chromosome is described as an assembly of a soft chromatin envelope associated with rigid elastic axes. It differs from the favored scaffold hypothesis that requires a proteinaceous core existing at all stages of the cell cycle and fulfilling the role of a skeleton that supports the chromatin loops (29). The model implies that the axes are not static structures but that instead they are assembled at mitosis upon the action of active condensin complexes as suggested in a recent, very comprehensive review (2). Such organization of the mitotic chromosomes will allow recombination to occur and the axes to be easily formed de novo.

Embryonic versus Somatic Chromosomes—Our experimental results clearly demonstrate a heterogenous structure of the mitotic chromosomes assembled in Xenopus egg extract. Poirier et al., however, have carried out stretching experiments on newt chromosomes by using the aspiration technique to catch the chromosome ends (12, 13). They have reported that these chromosomes could be described as homogenous elastic rods (13). This difference might reflect the fact that two different objects were studied by the two groups, i.e. somatic chromosomes isolated by micromanipulations of newt cells in one case and in vitro assembled chromosomes in extracts isolated from Xenopus eggs in the other. Indeed, the in vitro assembled chromosomes could be viewed as the very first embryonic Xenopus chromosomes. These chromosomes would be built in a way to accommodate the rapid divisions (30 min each) of the very early Xenopus embryo, i.e. they should be easier and more rapidly replicated and repaired. A reasonable way to do this is to keep the chromatin envelope very soft. However, during Xenopus development after the mid-blastula transition when the duration of the cell divisions is increased, the chromatin envelope might be made more rigid, and, in somatic cells, the chromosomes might thus be converted into homogenous objects. This conversion could be simply done by using agents that link the chromatin envelope. In other words, the somatic chromosomes could be viewed as matured embryonic chromosomes exhibiting a rigid chromatin envelope. If this is true, the stretch modulus of a newt chromosome should be the same when measured by using anti-histone antibody or anti-SMC antibody-coated pipettes.

If the above hypothesis for maturation of chromosomes during development is correct, the use of in vitro assembled chromosomes provides an excellent opportunity to study the structure of mitotic chromosomes. Indeed, only in this case is the mitotic chromosome structure “opened” for deeper understanding of the inner chromosome organization by using elasticity measurements.

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