Probing Chromosome Structure with Dynamic Force Relaxation

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We report measurements of the dynamics of force relaxation in single mitotic chromosomes, following step strains applied with micropipettes of force constant ~ 1 nN/μm. The force relaxes exponentially after an elongation (l/l_0) to less than $3\times$ native length, with a relaxation time ~ 2 sec. This relaxation time corresponds to an effective viscosity $\sim 10^5$ times that of water. We experimentally rule out solvent flow into the chromosome as the mechanism for the relaxation time. Instead, the relaxation can be explained in terms of the disentanglement dynamics of ~ 80 kb chromatin loop domains.

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Mitosis, the process by which eukaryote cells duplicate by division, involves structural transformation of chromosomes, from dispersed, transcriptionally active interphase chromatin fibers of ~ 30 nm thickness and $\sim$mm lengths, to compacted mitotic chromosomes ~ 1 μm in diameter and ~ 10 μm long. Mitotic chromosome structure has been studied for many years, yet remains poorly understood [1]. Microscopy [2–6] has not revealed a clear picture of *in vivo* mitotic chromosome structure, leading to proposals of different models of chromosome structure, including the organization of chromatin around a protein-rich "scaffold" [2], or alternately, hierarchies of helical folding [4].

An alternative to observation is study of material properties of mitotic chromosomes [7–11]. The force needed to deform animal chromosomes has been found to be ~ 1 nanonewton (nN) in microelasticity experiments, providing basic information about the strength of chromatin-tethering elements. Here we present the first characterization of the dynamics of stress relaxation in mitotic chromosomes. The relaxation rates are consistent with the dynamics of polymers tethered to and threaded through a polymer network [12].

We microdissected chromosomes from newt epithelial cells (TVI cell line) [13] grown in a monolayer on a small dish. Experiments were observed through the bottom of the dish using a 1.4 NA $60\times$ objective on an IX-70 microscope (Olympus). We selected mitotic cells between the end of prophase and metaphase, when the chromosomes are condensed and composed of two chromatids but not yet firmly attached to the mitotic spindle. Glass micropipettes with 2 μm inside diameter were used and positioned with motorized computer-controlled manipulators (Sutter, MP-285). A hole was made in the cell membrane by using one micropipette to spray a solution of 0.05% Triton in 60% PBS. Chromosomes then flowed out of the hole into the extracellular solution, allowing a chromosome to be grabbed with a second micropipette under ~ 10 Pa suction. After a few seconds of contact a chromosome became permanently stuck to the untreated glass of the micropipette. A third micropipette was then used to grab the other, free chromosome end. The result was a chromosome suspended between two micropipettes in the cell culture medium (Fig. 1).

Simple elasticity measurements are done by moving one pipette, while measuring the deflection of the second pipette. As has been shown for chromosomes from primary cultures of newt epithelial cells [9,11], TVI chromosomes show a linear elastic response for stretches up to $5\times$ native length, with a characteristic force of ~ 1 nN

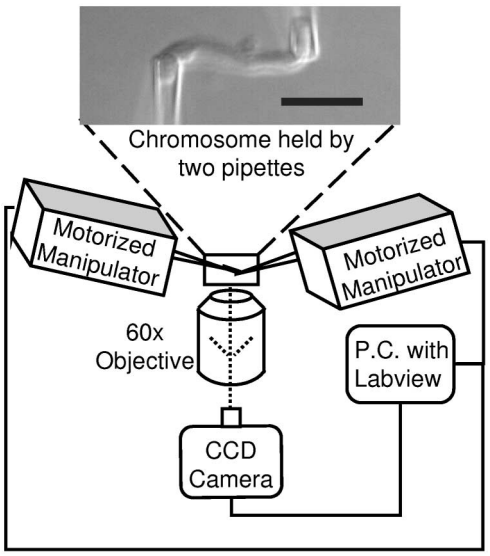

FIG. 1. Experimental setup used to study chromosome elasticity. Chromosomes are removed from cells, and then suspended in the cell medium between micropipettes, ~ 30 μm above the bottom of the culture dish. The micropipettes and chromosome are imaged through an inverted microscope objective. By slowly moving one pipette and observing the bending of the other pipette, a simple quasistatic force-extension experiment can be done. The same apparatus is used to carry out dynamical relaxation experiments, by rapidly stepping one pipette and then measuring the deflection of the other pipette as a function of time. This can be done at ~ 20 Hz using video frame capture. Pipettes with force constants of ~ 1 nN/μm are typically used, and are force calibrated following each experiment. Computer analysis gives 0.01 nN resolution. Bar = 10 μm.

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needed to double the length of a chromosome (Fig. 2 inset, solid curve). Previously we found that higher forces are observed during extension than retraction for strain rates faster than $\sim 0.1\ \mathrm{sec}^{-1}$ [11] indicating that mitotic chromosomes have a relaxation time on the order of 1 sec.

To quantify the stress relaxation, we performed dynamical experiments (Fig. 2). An isolated chromosome suspended between two micropipettes was rapidly stretched by stepping one pipette ($v = 100\ \mu\mathrm{m/sec}$) by 5 to 100 μm, while deflection of the other pipette was used to measure the dynamical force response. Using LABVIEW and IMAQ software (National Instruments), digitized video frames were acquired at 20 frames/sec. Step-relaxation cycles were done to 1.4, 1.7, 2, 2.4, 2.7, 3, 3.4, 3.7, 4, 4.4, 5.1, 5.8, 6.5, 7.2, and 7.9 times chromosome initial length. Two stretch-release cycles were done for each of these final lengths. In this paper, we focus mainly on results of one experiment; the same results were obtained from three separate runs on chromosomes from different cells. We also measured the time decay of the micropipettes by themselves to be below our time resolution of 0.05 sec (data not shown).

Figure 2 shows the dynamical response of a chromosome stretched to various lengths. The force-measuring pipette shows an initial force jump, followed by a decay to a smaller final force. The force decays (Fig. 3) have three

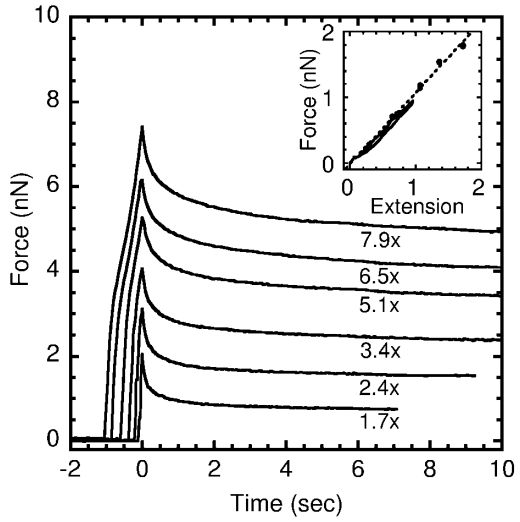

FIG. 2. Dynamics of force relaxation for a mitotic chromosome following step strains to lengths of 1.7, 2.4, 3.4, 5.1, 6.5, and 7.9 times initial length. The length change is listed just below the corresponding time series. Initial force jumps are followed by force decays to a final force. For the longer extensions, only part of the decay curves are shown. Inset: Force (nN) vs extension (change in length in units of initial length) derived from the final extensions observed following the step strains (points), which have a linear fit of $y = (1.1\ \mathrm{nN})x$ (dashed line). Before the step strain experiments a quasistatic extension-retraction experiment was done on the same chromosome (solid curve). There is excellent agreement between the two force-extension results, showing that up to 2× extensions, the chromosome reaches its equilibrium elongation in the step-strain experiments.

important features. First, an initial decay of ~0.5 sec is observed for each elongation (note elongate refers to length divided by initial length). Second, for an elongation less than 3 times initial length (3×), the initial decay is followed by an exponential decay with decay time ~2 sec. Third, after being rescaled in force, the decays after extensions of <3× are the same (note that ○ and □ show different extensions in Fig. 3). This superposition and final exponential decay is characteristic of linear elastic response.

The viscous relaxation time of a chromosome of length $l_0 \sim 10\ \mu$m attached to the pipette with spring constant $k \sim 1\ \mathrm{nN}/\mu\mathrm{m}$ with effective viscosity η, is $\tau \sim \eta l_0/k$. This implies an effective viscosity $\eta \sim 100\ \mathrm{kg/(m\,sec)}$, about 10^5 times that of water. As expected, the relaxation dynamics are unrelated to viscous flow of the buffer past the chromosome.

For jumps to >3×, the duration of the decay gradually increases (Fig. 3, inset). A permanent increase in the relaxed chromosome length following release of applied stress coincides with this increase in the decay time (data not shown), indicating that irreversible damage occurs during jumps to >3× initial length. This is consistent with quasistatic elongations beyond 5× being irreversible [10,11]. In the irreversible regime, the terminal force relaxation no longer fits an exponential (Fig. 3). For the longer steps to >6× initial length, force ~const − ln(time) describes the entire decay range,

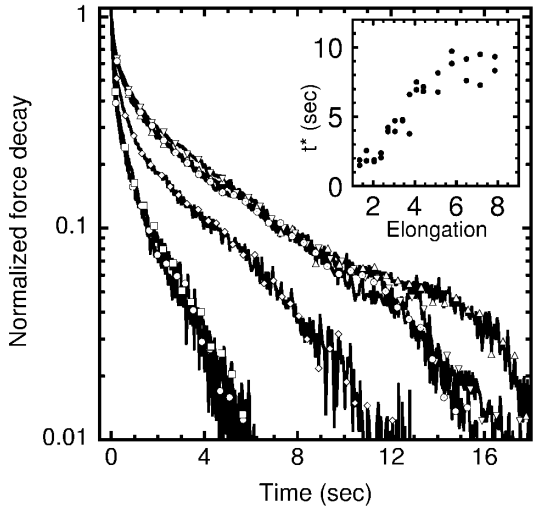

FIG. 3. Scaled force relaxation data (force − final force)/(initial force after step − final force) is plotted so that the stress decays from 1 to 0. The plots correspond to the extension shown in Fig. 2 of 1.7 (○), 2.4 (□), 3.4 (◇), 5.1 (△), 6.5 (▽), and 7.9 (⊙); every twentieth data point is shown. The decays all follow roughly the same behavior to 50% of the transient force has relaxed. This is followed by a slower decay, which increases for extensions beyond 3×. Inset: The time in which 90% of the transient force has decayed, t^*, versus elongation (length in units of the initial length). The low-extension curves in the reversible regime show a final exponential decay with a lifetime comparable to t^*. The higher-extension results show a nonexponential final decay.

suggestive of a broad range of free energy barriers to large extensions which are crossed thermally as failure occurs.

Following each step we determined the final force versus extension after the decays (Fig. 2 inset, points). The resulting force-extension behavior is linear to 3× initial length (note Fig. 2 shows change in length in units of initial length) with a slope of 1 nN in accord with previous results for chromosome elasticity [9–11]. The force-extension curve derived from the step experiments matches the result of a quasistatic force-extension measurement done just before the dynamic experiments (Fig. 2 inset, solid curve).

One explanation for the relaxation time scale of 2 sec might be a slow force equilibration over the length of the chromosome. However, the stationary pipette responds within 0.05 sec to the step of the other pipette, and the decays of both pipettes following the step extension overlap (data not shown). Therefore stress is supported uniformly throughout the chromosome throughout the decay.

Another explanation for the scale of the relaxation time in the reversible, linear relaxation regime (<3× final length) might be the squeezing of fluid through interstices between chromatin fibers, as would occur in a gel following a step-strain [14]. Such flow certainly occurs because chromosomes remain nearly the same diameter even when doubled in length: the Poisson ratio of a mitotic chromosome is ~0.1 (11). Chromosomes thus appreciably increase in volume when stretched, requiring an inflow of fluid. In separate experiments we simultaneously measured chromosome width and force relaxation. Chromosome width reaches its final value within 0.05 sec of being stretched (Fig. 4). This is during the early stages of the force relaxation, so all bulk flow into the outer region of the chromosome is finished well before the transient stress has decayed.

Having ruled out inhomogeneous relaxation and solvent flow, the observed slow stress relaxation for an elongation <3× must be due to reorganization of chromosome structure at scales much smaller than the chromosome length. We see two possibilities: first, we may be breaking crosslinks (bonds) between chromosome fibers [Fig. 5(IIIa)]. However, a crosslink-breaking picture suggests that there should be either irreversibility for small <2× strains, or a slow "healing" process following each stretch-release cycle, as the crosslinks find their partners and relink. Instead of this, we find that chromosomes return to their native lengths immediately as stress is removed.

A second and more plausible explanation for the stress relaxation is based on entanglement dynamics. Any scheme of chromosome folding must include two features: each half of a mitotic chromosome is made of a single, long (few cm in our case) chromatin fiber; and those fibers must be somehow attached to themselves to keep the chromosome compacted. This implies the existence of "ends" or "loop domains" [Fig. 5(II)], which have in fact been observed in a number of ways [2,3]. Such loops will

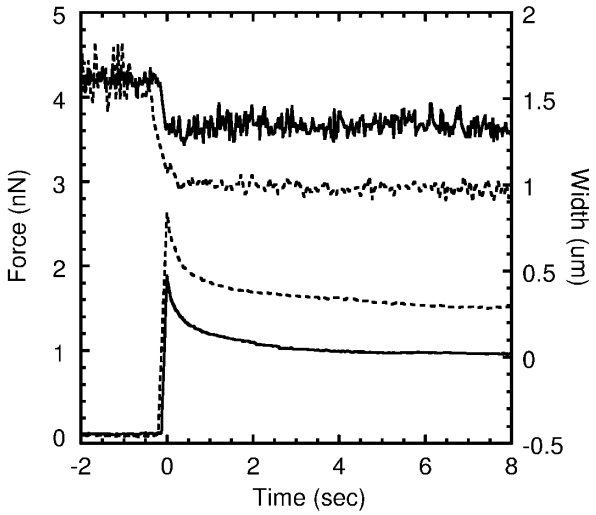

FIG. 4. Comparison of force (lower curves) and width relaxation (upper curves) for a mitotic chromosome following a step strain to 1.8 (solid) and 2.2 (dashed) times native length. Force and width were simultaneously measured at a ~20 Hz rate by analysis of digitized video images. Because of inhomogeneities in chromosome width, the width was averaged over 1 μm lengths, for which the chromosome width is constant. One width section for each extension is shown; each width section equilibrated in less than 0.05 sec. The width and therefore chromosome volume is seen to equilibrate on a time scale short compared to the force. This indicates that the force relaxation is not due to the hydrodynamics of liquid being squeezed out of the chromosome by the applied stress, and is therefore due to reorganization of the chromosome fibers themselves [18].

behave as polymers tethered inside a polymer network. Following a step strain, they will be affinely stretched, contributing a large transient stress. This stress will then relax as the extended loops pull out of the chromosome region in which they were originally embedded. Following stress release, the loop domains will re-embed themselves into the relaxed chromosome by conformational diffusion.

We can estimate the time scale for this process by considering one loop, which initially will be in a random-walk-like conformation. A step strain of the chromosome will affinely deform the loop as well as the surrounding network in which it is embedded. Some of the stress contributed by the deformed loops can relax if they can regain a random-coil conformation. However, a loop can do this only by the torturous process of transfer of its entire length through the network 'pore' near its base [Fig. 5(IIIb)]. In the small-deformation regime this process requires the loop end to diffuse along the path defined by its initial conformation [15]. This process requires a time of roughly $\tau_0\, e^{\alpha N}$ for a loop which is N segments long, where τ_0 is a chromatin persistence-length relaxation time of roughly 1 μsec, and where α is a order-unity constant. Assuming the relevant fiber is chromatin, we can estimate N from previous studies. Electron micrograph studies of histone depleted metaphase chromosomes found loop domains of about 80 kb of DNA [2,3]. The length of chromatin fiber containing 80 kb is at least 0.5 μm since DNA is

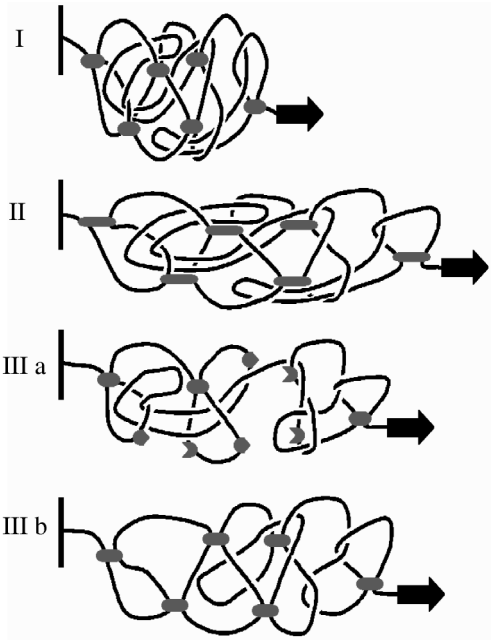

FIG. 5. Models of stress relaxation inside a mitotic chromosome. A chromosome consists of a long (10 cm for TVI cells) chromatin fiber, tethered to itself to form a compact mitotic chromatid (I). To form such a structure, there must be "loop domains" which will be entangled with the surrounding chromatin. Immediately following a step strain, the network and any loop domains entangled with it will both be stretched (II). Stress relaxation can then take place either by the breaking of network crosslinks (IIIa), or by the reorganization of loop domains (IIIb). Our data suggest that crosslink breaking occurs only for step strains beyond 3× elongation which leads to irreversible chromosome stretching. Loop-domain reorganization is left as the main possibility for the slow relaxation we observe for step strains to less than 3× elongation.

compacted into chromatin by up to 50 times [17]. A length of 0.5 μm has about 15 segments, since chromatin has a persistent length of $\sim$30 nm [16]. Therefore, τ is on the order of seconds, which is consistent with the measured decay time.

We can understand why the decay is initially fast and then slows down to a final exponential decay in terms of this model, since part of the transient force can be relaxed quickly by partial changes of loop conformations (e.g., extension of only slightly constrained chromain segments). However, for all of the transient force to relax, topological barriers must be crossed, requiring a wide range of conformations to be explored, giving a slow final decay. The slowing down of stress relaxation for larger strains can also be understood, in terms of increased friction encountered by loops stretched into tight contact with the surrounding network. At even higher strains, some loops or surrounding network crosslinks will break, leading to the irreversibility observed for steps to $>3\times$ initial length.

In conclusion, we have found that mitotic chromosomes quickly stretched display well-defined elastic response with a relaxation time on the second time scale. We find that this relaxation is not due to gel-draining dynamics, but is instead most likely due to the relaxation of chromosome 'loop' domains containing up to 80 kb of DNA. While the observation of loop domains by dynamical relaxation of chromosomes is indirect and very rough, our study indicates that loose loop domains are present in physiological conditions. In addition, our results suggest that mitotic chromosomes are not folded in the precise fashion of globular proteins or other biopolymers organized by sequence-specific interactions. Instead we are led to a model of relatively loosely self-tethered chromatin, which admits rapid flow of small molecules in and out of the chromosome volume, and permits large chromatin domains to change conformation by slowly sliding between their neighboring fibers.

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