Supporting Information

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SI Text

For the stem and flower regime, a universal scaling method has been developed by Manneville [Manneville S, Cluzel P, Viovy JL, Chatenay D, Caron F (1996) *Europhys Lett* 36:413–418] that allows for the comparison of polymer chains of varying contour length and persistence lengths relaxing in fluids of different viscosities. In this approach, the length of the retracting polymer is divided by the half-length and the time by the half-time (length of time taken for polymer to retract to its half-length). The expected slope of 0.5 (see *Introduction*) has been investigated by others, with their experimental data showing a scaling exponent of 0.51 [Manneville S, et al. (1996) *Europhys Lett* 36:413–418]. If we do not account for the present 2 different relaxation regimes, and instead analyze our entire data set using the stem and flower model, we see deviations from the expected scaling as seen in Fig. S2. As a result, data collected from our in vivo experiments were analyzed using both the taut and stem and flower models. This approach was then verified using our in vitro system to show that it is reasonable to use these conformations to extract information about the viscosity of the surrounding environment and the tension force applied to the polymer immediately before the relaxation event.







Fig. S2. Complete, rescaled data sets for in vivo, in vitro (high viscosity), and in vivo (low viscosity) relaxation events. Both the in vivo and the high viscosity in vitro data sets show multiple slopes, indicating the presence of more than 1 scaling regime.

DNA C



Fig. S3. Taut analysis parameter space showing the change in force as a function of viscosity for a range of persistence lengths (persistence length in microns). Here the polymer length and the relaxation time obtained from the Chromatin 1' data set, 3.4 μ m and 167 s, respectively, were held constant.



Fig. S4. The absorption of high energy light causes the DNA molecule to rupture, allowing the relaxation process to be imaged.

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Fig. S5. In vivo chromatin relaxation montage shown in Fig. 4A without image processing for the removal of out-of-plane fluorescent structure.

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Movie S1. In vivo relaxation of chromatin stretched by the microtubule-based spindle.

Movie S1

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Movie S2. In vitro relaxation of dsDNA in a high-viscosity environment.

Movie S2

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