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As a Nucleus Enters a Small Pore, Chromatin Stretches and Maintains Integrity, Even with DNA Breaks

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ABSTRACT As a cell pushes or pulls its nucleus through a small constriction, the chromatin must distort and somehow maintain genomic stability despite ever-present double-strand breaks in the DNA. Here we visualize within a living cell the pore-size dependent deformation of a specific locus engineered into chromosome-1 and cleaved. An mCherry-tagged nuclease targets the submicron locus, causing DNA cleavage and recruiting repair factors such as GFP-53BP1 to a large region around the locus. Aspiration of a cell and its nucleus into a micropipette shows that chromatin aligns and stretches parallel to the pore. Extension is largest in small pores, increasing >10-fold but remaining 30-fold shorter than the DNA contour length in the locus. Brochard and de Gennes' blob model for tube geometry fits the data, with a simple modification for chromatin crowding. Continuity of the highly extended, cleaved chromatin is also maintained, consistent with folding and cross bridging of the DNA. Surprisingly, extensional integrity is unaffected by an inhibitor of the DNA repair scaffold.

Cells have been seen to squeeze through small gaps of matrix and other cells in many basic processes that range from immune surveillance to disease, and include invasion of cancer cells into nearby tissue or entry into blood capillaries. The nucleus is the largest and stiffest organelle in the cell (1) but a cell can often push, pull, and forcibly distort this chromatin-filled organelle through a constriction (2,3). Pulling a flexible polymer into a tube is a classic problem in polymer physics (4), but any relevance to chromatin within a nucleus that is being pulled through a pore is unclear, particularly given the crowding estimated as ~70% chromatin volume fraction (5). Also unclear are the effects or not of double-strand breaks in the DNA backbone of chromatin, although such breaks—which seem to be present at low levels in all cells (6)—have been speculated to be enhanced by cell migration through small pores (2). When cleaved DNA is stretched by optical traps in single molecule studies, it is held together by a scaffold of repair factors (7), but chromatin is made up of many other cohesion-enhancing proteins, which motivates stretching of cleaved chromatin in intact nuclei of living cells.

A repetitive locus in one arm of chromosome-1 has been engineered previously into the U2OS osteosarcoma cell line for live cell imaging of the locus (8) (Fig. 1 *A*). The ~200 repeats of the lactose operon (LacO) add up to a DNA contour length >600 μm. In methanol-fixed cells, all of chromosome 1 can be seen by fluorescence in situ hybridization, revealing a fractal shape microns in size, indicative of chromatin folding (Fig. 1 *B*). In live cells, the locus can be seen by inducing expression of a fusion protein of lactose repressor (LacR)-mCherry-FokI nuclease. LacR binds to the LacO sequence, and FokI causes DNA breaks upon binding, based on accumulation of a DNA damage response protein, GFP-53BP1, at the locus and around it (Fig. 1 *C*). A histone that is modified upon DNA damage, γH2AX, also occupies the same large area as part of a repair scaffold complex (9,10). DNA cleavage by FokI nuclease is further supported by evidence of enhanced DNA synthesis at the locus, even though locus size appears unaffected by breaks and processes they activate (Fig. S1 in the Supporting Material).

The deformation dynamics of a specific chromosomal locus within an interphase nucleus have not been visualized previously, although the micromechanics of isolated mitotic chromosomes have been studied (11), as has the stretching of mitotic chromosomes in live yeast (12). To controllably deform and visualize the cleaved chromatin

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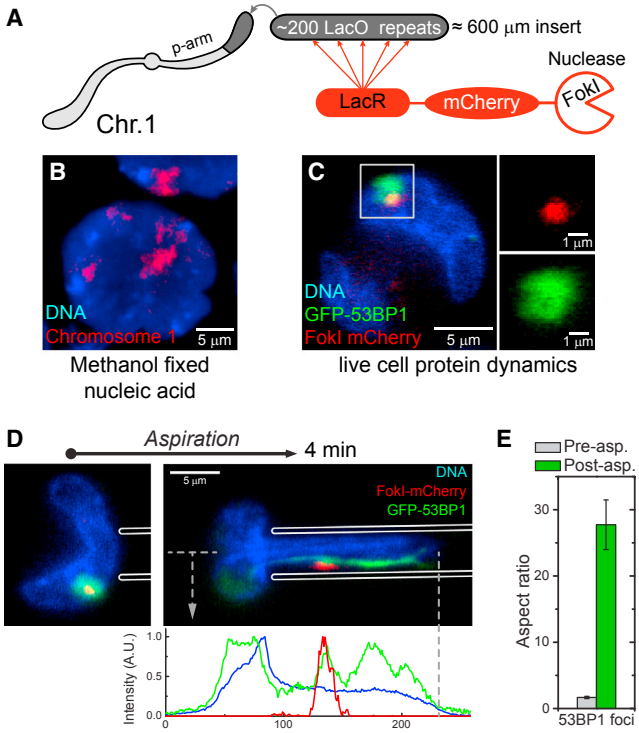

FIGURE 1 Chromosome-1 locus seen in live cells pulled into a micropipette. (*A*) The induced fusion protein has mCherry to visualize, LacR to bind LacO repeats in chromosome-1 DNA, and also FokI nuclease to cleave DNA in the locus. (*B*) Chr-1 as visualized by fluorescence in situ hybridization in fixed, dehydrated cells. (*C*) Colocalization of mCherry fusion protein with repair protein GFP-53BP1. (*D* and *E*) Within a nucleus in a micropipette, GFP-53BP1 extends and aligns, with an aspect ratio that increases >10-fold (n = 11 cells, N = 3 experiments).

within a living cell's nucleus, cells were detached from their substrate, their cytoskeleton was disassembled with latrunculin, and the intact cell was aspirated into a micropipette of diameter ~2.5–4.0 μm (13). Cells were studied within ~1 h of preparation. As the nucleus squeezes into the micropipette, the chromatin-bound GFP-53BP1 foci always align and stretch in the axial direction, increasing the aspect ratio >10-fold (Fig. 1 *D*). These observations begin to suggest that nuclear deformation affects chromatin organization.

The mCherry-labeled locus always stretches less than the repair scaffold of GFP-53BP1, but it also aligns as it enters the constriction (Fig. 2 *A*), adjacent to many other chromosomes pulled in parallel into the pore (i.e., blue DNA). The estimated diameter profiles of stretched FokI foci are close to the averages listed in Table S1, even though the profiles are calculated from just the initial width and the intensity profile (Fig. 2 *A*). A maximum stretched length of ~14 μm (Table S1) is <3% of the DNA contour length and thus indicates a high degree of chromatin folding. The foci stretch most upon entry and partially shorten as they displace further into the pore (Fig. S2). Convergent flow into such a constriction will stretch any small piece of fluid or solid material provided it is not overly compressible (Fig. S2). However, because the tube diameter remains constant after entry, the stretch should remain constant unless the material is more complex—as seems applicable here with recoil and reequilibration of the locus and the other chromatin in the pore.

The DNA damage response involves many factors, including ATM kinase that phosphorylates many targets (such as histone H2AX to produce γH2AX) in the recruitment of DNA repair proteins like 53BP1 (14). The ATM inhibitor KU55933, or ATMi, significantly attenuates γH2AX at the locus (Fig. S3) (15). Despite such attenuation of the repair scaffold, the mCherry foci stretch the same for ATMi treated cells and for not-treated cells—at least when comparing aspect ratio of foci aspirated into micropipettes of similar diameter (Fig. 2 *B*). Although the aspect ratios of stretched foci show no obvious correlations with either their original locations or their original sizes (Fig. S4), aspect ratios increase with decreasing pipette diameter. A statistically significant increase in

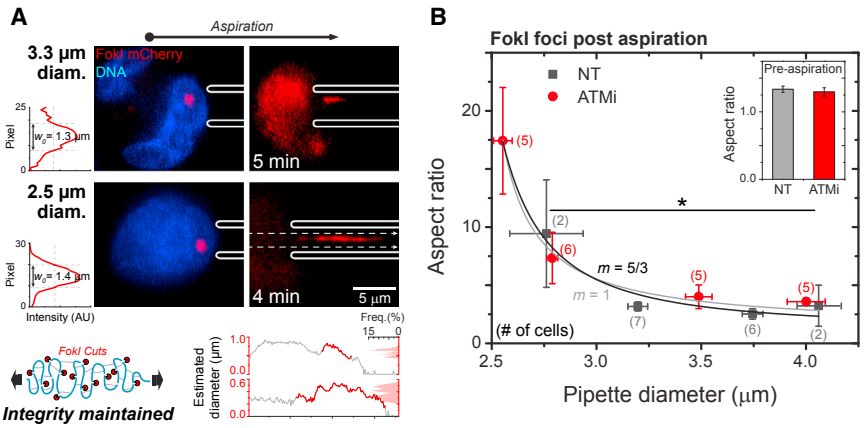

FIGURE 2 (*A*) FokI-mCherry focus also aligns and extends parallel to the pipette constriction. The diameter of preaspirated foci (w_0) at full width half-maximum was divided by the square-root of the cross-section intensity and multiplied by square-root of the intensity profile of stretched chromatin to calculate the diameter profile (*lower plots*). Integrity of chromatin is maintained. (*B*) Inhibition of DNA damage response by ATMi does not enhance FokI stretching. Data for ~2.8 and ~4 μm pipette diameter were pooled and show a significant change of aspect ratio with pipette diameter (*p = 0.05). (*Inset*) Aspect ratio of FokI foci before aspiration at ~1.3 μm (n = 17–21 cells, N > 3 experiments).

aspect ratio of foci also requires pore diameters smaller than ~3 μm.

A scaling picture first used to describe conformational changes of a single, self-avoiding chain confined to a tube or a pore (4,16) has been examined in simulations (17) and modified for soft walls (18). In simplest form, for a pore diameter d, the aspect ratio λ of a single chain is calculated from the parallel dimensions of the chain R_{par} divided by the perpendicular dimension R_{perp}, which gives $\lambda = R_{par} / R_{perp} \sim 1 / d^{5/3}$ when ignoring constant prefactors. The locus here is surrounded by chromatin so that the pore constricting the locus is much smaller than the pipette diameter, i.e., $d = (D_{pip} - c)$. Also, outside a pore ($D_{pip} \rightarrow \infty$), λ has the preaspiration aspect ratio ($a = 1.3$). Thus

$$\lambda = a + b/(D_{pip} - c)^m, \quad (1)$$

where $m = 5/3$ according to the blob theory. A good fit with $m = 5/3$ is achieved ($R^2 = 0.97$: $b = 2.9$, $c = 2.2$ μm), with other values of m also giving reasonable fits (Table S2). All data points (not-treated and ATMi) were fit because inhibition of the DNA damage response does not alter the trend in aspect ratio (i.e. nuclease dominates here).

Importantly, even though DNA within the engineered locus is constantly cleaved by nuclease, intensity profiles of mCherry indicate continuity so that integrity of the chromatin is maintained within a distended nucleus regardless of whether repair is inhibited (Fig. 2 *A*, *sketch*). Such locus integrity is consistent (in intact nuclei) with a highly folded structure being maintained by force-resistant chromatin cohesion factors (19).

In this Letter, we sought to show under native conditions of an interphase nucleus in a living cell that constriction-induced nuclear deformation orients and stretches a well-defined, cleaved chromatin locus parallel to the pore axis. Stretching is significant for a pore with a critical diameter <3 μm, but of course such a large pore is also constricting many nearby chromosomes within a crowded nucleoplasm. Potentially, chromatin compaction by small constrictions increases the volume fraction to a critical value, thus decreasing the accessible space within the nucleus (20) and, in turn, increasing the chromatin stretching. Importantly, the integrity of the stretched and cleaved locus is always maintained, which strongly indicates that constrictions are unlikely to provide sufficient stress to mechanically break the covalent bonds in both strands of DNA. Distortion and/or displacement of nuclease cleaved DNA could, however, disturb repair processes. This could be important because DNA misrepair can contribute to genomic instability, including insertions, deletions, or perhaps point mutations, all of which might contribute to cancer progression (21). For this reason and more, it is certainly worth clarifying what is happening within the multiple blobs of chromatin (Figs. 2 *A* and S2) that are caused by the extension of the single starting locus (Fig. 1 *C*).

SUPPORTING MATERIAL

Supporting Materials and Methods, four figures, and two tables are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(16)30881-5.

AUTHOR CONTRIBUTIONS

J.I., Y.X., and C.R.P. designed research, performed research, and analyzed data; R.A.G. contributed analytic tools; and D.E.D. analyzed data and wrote the article with J.I.

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