CHROMOSOMES

Mitotic chromosome assembly despite nucleosome depletion in *Xenopus* egg extracts

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The nucleosome is the fundamental structural unit of eukaryotic chromatin. During mitosis, duplicated nucleosome fibers are organized into a pair of rod-shaped structures (chromatids) within a mitotic chromosome. However, it remains unclear whether nucleosome assembly is indeed an essential prerequisite for mitotic chromosome assembly. We combined mouse sperm nuclei and *Xenopus* cell-free egg extracts depleted of the histone chaperone Asf1 and found that chromatid-like structures could be assembled even in the near absence of nucleosomes. The resultant "nucleosome-depleted" chromatids contained discrete central axes positive for condensins, although they were more fragile than normal nucleosome-containing chromatids. Combinatorial depletion experiments underscored the central importance of condensins in mitotic chromosome assembly, which sheds light on their functional cross-talk with nucleosomes in this process.

The nucleosome, which consists of two copies each of the four core histones (H2A, H2B, H3, and H4) and DNA wrapped around them, represents the first hierarchy of folding of eukaryotic chromatin (*1*). Immediately before cell divisions, large-scale folding is imposed on chromatin, and this process, known as mitotic chromosome assembly, ensures faithful segregation of duplicated chromatids into daughter cells (*2*, *3*). The core histones accounts for approximately half a weight of the whole-protein components constituting mitotic chromosomes (*4*, *5*). The second abundant chromosomal proteins are the multiprotein complexes termed condensins (*6*, *7*), which have been shown to be essential for building mitotic chromosomes in various organisms (*8*–*11*). It remains largely unknown, however, to what extent the core histones directly contribute to mitotic chromosome assembly, or how condensins act on nucleosomal fibers (*12*, *13*).

To explore a potential role of histones in mitotic chromosome assembly, we exploited a cell-free system made from *Xenopus laevis* eggs (*14*). In a conventional protocol, *Xenopus* sperm nuclei incubated with metaphase-arrested egg extracts are transformed into clusters of single chromatids, independently of transcription or DNA replication (*5*). However, because histones H3 and H4 that are derived from *Xenopus* sperm nuclei persist on chromatin throughout the assembly reaction (*11*), it is virtually impossible to manipulate this pair of histones. To overcome this limitation, we sought to introduce mouse sperm nuclei, which barely retain histones (*15*), into *Xenopus* egg extracts.

We first established a protocol for preparation of mouse sperm nuclei that could be used as substrates in the cell-free assay (Fig. 1A). When the resultant mouse sperm nuclei were incubated with a mitotic extract, they quickly lost their original teardrop-like configuration and turned into fibrous structures within 5 min. Then, the chromatin fibers gradually thickened and untangled, being converted into rod-shaped mitotic chromatids by 180 min. The morphology

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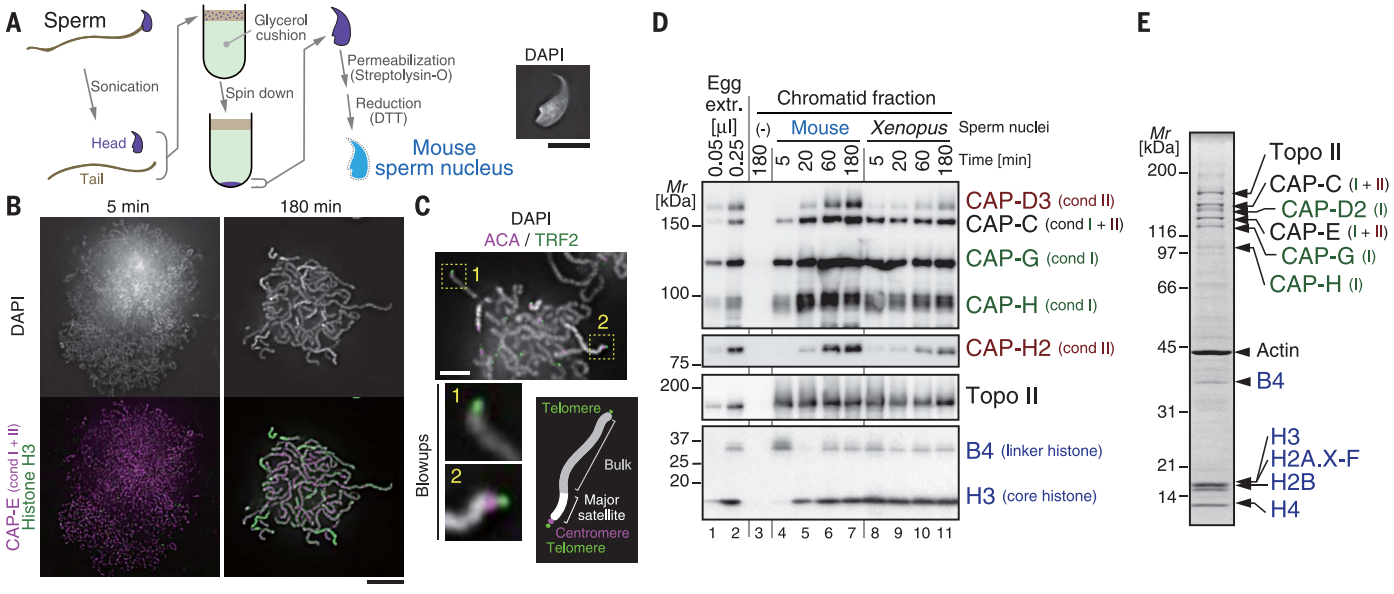

Fig. 1. Mouse sperm nuclei can be converted into mitotic chromatids in *Xenopus* egg extracts. (**A**) Preparation of mouse sperm nuclei. (**B**) Immunofluorescence of mouse sperm nuclei incubated with a *Xenopus* egg extract. At the indicated time points, chromatin was labeled with anti-CAP-E, antihistone H3, and DAPI. (**C**) Centromeres and telomeres were labeled with anti-centromere antibody (ACA) and anti-TRF2, respectively. Presumed structure of a mouse sperm-derived chromatid is depicted in the illustration. (**D**) Immunoblotting of proteins that associate with mouse or *Xenopus* sperm nuclei incubated with egg extracts. (**E**) Protein composition of mitotic chromatids assembled from mouse sperm nuclei in *Xenopus* egg extracts. The gel was stained with Coomassie blue. Scale bars, 5 µm.

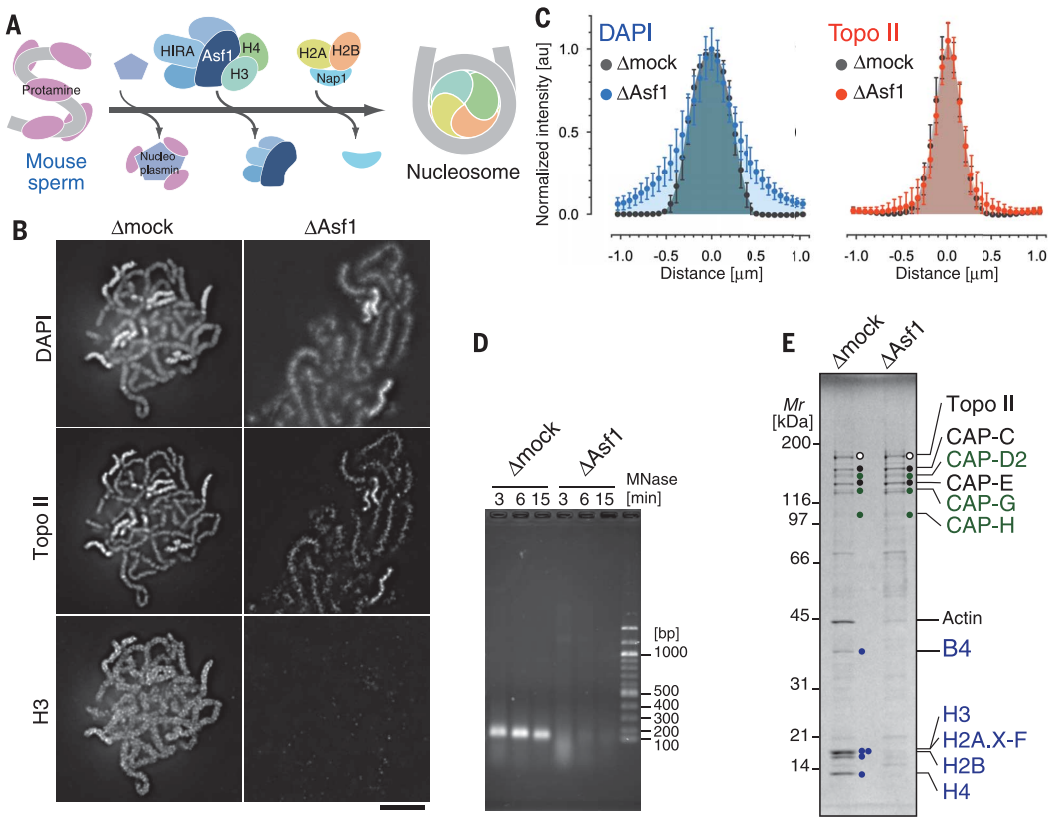

Fig. 2. Asf1 depletion impairs nucleosome assembly. (**A**) Putative scheme for nucleosome assembly on mouse sperm DNA. (**B**) Immunofluorescence of mouse sperm nuclei incubated with Δmock or ΔAsf1 extracts for 180 min. Chromatids were labeled with anti-topo II, antihistone H3, and DAPI. (**C**) Line profiles of normalized signal intensities of DAPI and anti-topo II. Lines drawn perpendicular to chromatid axes were analyzed. The mean and SD of 20 lines are shown. (**D**) Micrococcal nuclease (MNase) digestion assay of chromatids assembled in the indicated extracts. The gel was stained with ethidium bromide. (**E**) Protein compositions of mitotic chromatids assembled in the indicated extracts. The gel was stained with Coomassie blue. Scale bar, 5 μm.

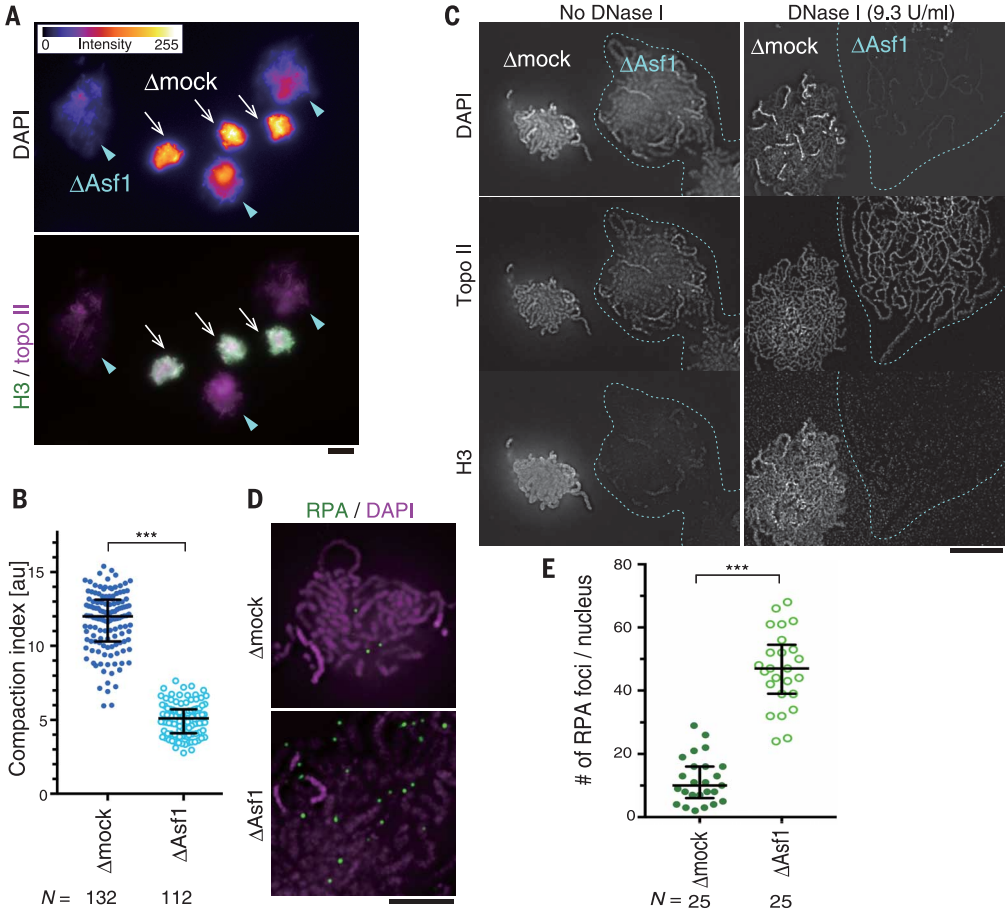

Fig. 3. Nucleosome-depleted mitotic chromatids have sparse and fragile chromatin loops. (**A**) Direct comparison of clusters of chromatids assembled in Δmock and ΔAsf1 extracts. Fixed samples derived from the two different reactions were spun onto a single coverslip and processed for immunofluorescence. (Top) Distribution of the intensity of DAPI signals. (Bottom) Immunofluorescence for H3 and topo II. Nucleosome-containing and nucleosome-depleted chromatids are indicated by the arrows and arrowheads, respectively. (**B**) The compaction indexes (the average DAPI intensities per unit area) were plotted. (**C**) DNase I digestion assay. Fixed chromatids [prepared as in (A)] were treated with or without the nuclease. (**D**) Immunofluorescence for RPA. (**E**) The numbers of RPA foci per each nucleus were plotted. Bars indicate median and interquartile ranges. ***$P < 0.0001$, Mann-Whitney U test, (B) and (E). Scale bars, 10 μm.

of the final products was remarkably similar to that derived from *Xenopus* sperm nuclei (Fig. 1B and fig. S1), except that the chromatids derived from mouse sperm nuclei exhibited acrocentric structures, each of which had a 4′,6-diamidino-2-phenylindole (DAPI)–dense, major-satellite DNA region at one of the tips (Fig. 1C). Although histone H3 was detectable at much later time points on mouse sperm DNA than on *Xenopus* sperm DNA (Fig. 1D and fig. S1), the protein compositions of the final products that were derived from the two different substrates were similar to each other (Fig. 1E) (*5*). We also confirmed that condensins and topoisomerase II (topo II) were indispensable for chromatid assembly derived from mouse sperm nuclei (fig. S2), as had been demonstrated with *Xenopus* sperm nuclei (*8*, *14*, *16*).

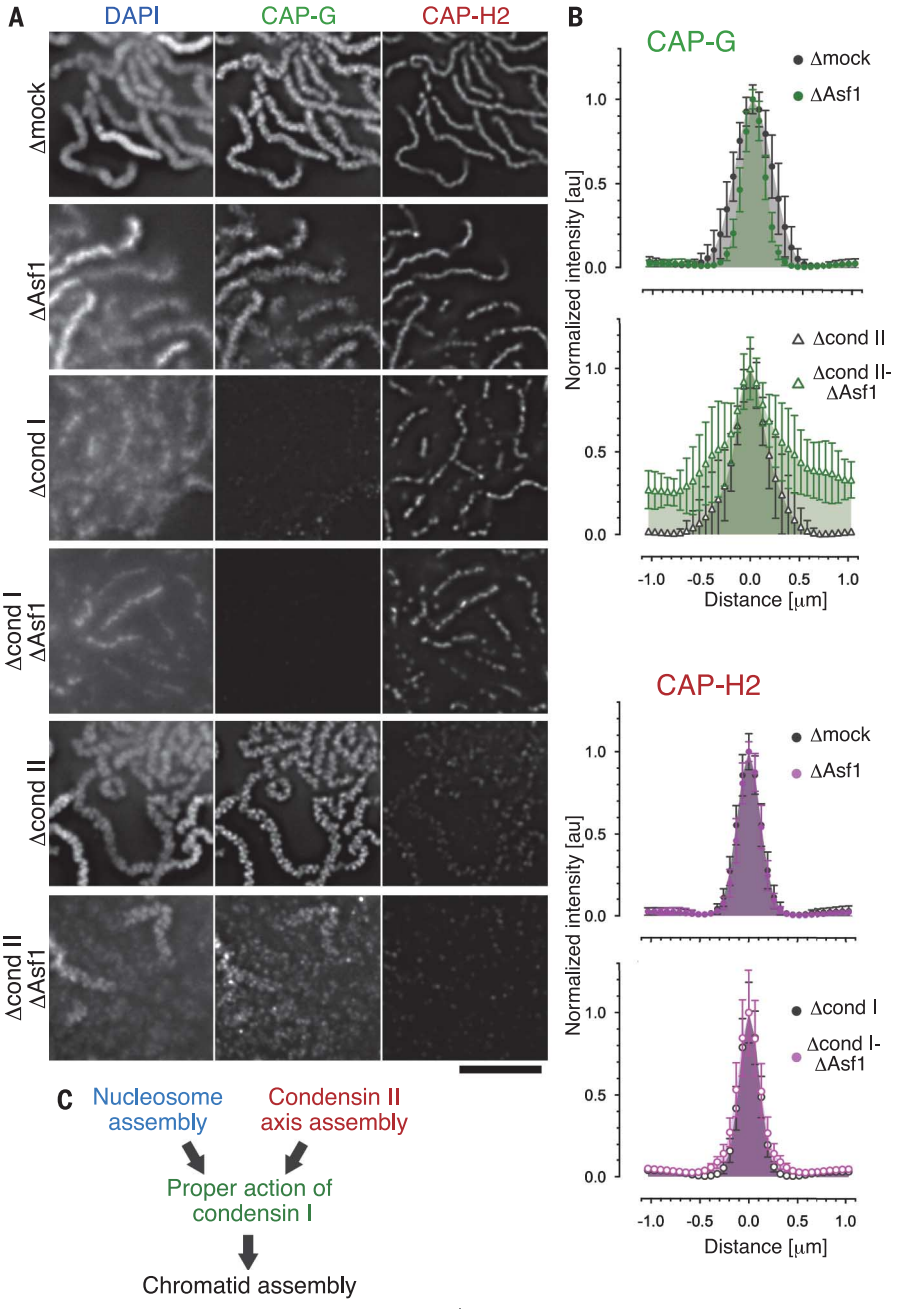

Fig. 4. Functional cross-talk between condensins and nucleosomes is vital for full compaction of chromatids. (**A**) Blow-up images of mitotic chromatids assembled in the egg extracts indicated. The resultant chromatin was labeled with anti-CAP-G (condensin I) and anti-CAP-H2 (condensin II). (**B**) Line profiles of normalized signal intensities of CAP-G and CAP-H2. The mean and SD of 20 lines are shown. (**C**) A model for mitotic chromosome assembly proposed in the current study. Scale bar, 5 μm.

De novo nucleosome assembly on mouse sperm DNA in fertilized eggs is mediated by sequential actions of multiple histone chaperones (Fig. 2A) (*17*–*19*). We reasoned that the same set of histone chaperones would work on mouse sperm DNA incubated with *Xenopus* egg extracts, and that Asf1 would play a pivotal role in deposition of H3 and H4 after protamine removal. To inhibit this step, we depleted more than 95% of Asf1 from egg extracts (fig. S3A). As expected, the resultant Asf1-depleted (ΔAsf1) extracts did not support nucleosome assembly on a naked circular DNA (fig. S3B) (*20*). However, ΔAsf1 extracts supported normal chromatid assembly starting from *Xenopus* sperm nuclei (fig. S4) because these substrates retain histones H3 and H4 and do not require Asf1 for nucleosome assembly (*11*).

We then tested what would happen when mouse sperm nuclei were incubated with ΔAsf1 extracts. If nucleosome assembly was indeed essential for subsequent large-scale folding of chromatin, then no mitotic chromatid-like structures would be produced under this condition. To our great surprise, however, we found that structures reminiscent of mitotic chromatids appeared after a 180-min incubation (Fig. 2B). Unlike the well-structured, rod-shaped chromatids assembled in mock-depleted (Δmock) extracts, the structures assembled in ΔAsf1 extracts looked anomalous, being composed of the two distinct parts: a DAPI-dense central axis enriched with topo II and hazy chromatin masses surrounding it. Line scan profiles perpendicular to the axis revealed a broader distribution of DNA in the chromatids assembled in ΔAsf1 extracts than those assembled in Δmock extracts but detected no sizeable differences in topo II distributions between the two sets of chromatids (Fig. 2C). Virtually no signal of histone H3 was detectable in the chromatids assembled in ΔAsf1 extracts (Fig. 2B). When a micrococcal nuclease digestion experiment was performed, mononucleosome-sized DNA fragments resistant to the treatment were detected in the chromatids assembled in Δmock extracts but not in those assembled in ΔAsf1 extracts (Fig. 2D). Protein analyses demonstrated that both core and linker histones were nearly absent in the latter fractions, whereas almost an identical set of nonhistone proteins were recovered in the two chromatin fractions (Fig. 2E, fig. S5, and tables S1 and S2). Nucleosome-independent binding of condensins to DNA observed here was contradictory to some early studies (*21*, *22*) but was consistent with more recent results from a cell-free assay that combines DNA-coupled beads and histone-depleted egg extracts (*23*) and other various approaches (*11*, *24*, *25*). Although our results demonstrated that mitotic chromatid-like structures can be assembled even in the near absence of nucleosomes, the possibility cannot be excluded that a trace amount of histones makes a minor yet non-negligible contribution to assembling these structures.

We then compared structural properties of the nucleosome-depleted (ΔAsf1) and nucleosome-containing (Δmock) chromatids in detail. The

relative position of centromeres and telomeres within the chromatids was not largely affected upon nucleosome depletion (fig. S6). Clusters of the nucleosome-depleted structures spread into larger areas and displayed a lower DAPI intensity per unit area than those of the nucleosome-containing chromatids in microscopic specimens (Fig. 3, A and B). We also found that DNA within the nucleosome-depleted structures was more sensitive to deoxyribonuclease I (DNase I) than that within the control chromatids (Fig. 3C and fig. S7). This tendency was more prominently observed in bulk DNA than in major satellite DNA. The number of foci positive for replication protein A (RPA), a single-stranded DNA binding protein complex, increased upon nucleosome depletion (Fig. 3, D and E), implicating that these chromatids contain anomalously unwound DNA or damaged DNA under repair at a high frequency. Thus, the nucleosome-depleted chromatids were sparser and more fragile than the nucleosome-containing chromatids.

Last, we investigated functional contributions of condensins to chromatid assembly and their dependency on nucleosomes. To this end, we prepared extracts depleted of Asf1, condensin I, or condensin II, either individually or in combination, and then incubated mouse sperm nuclei with those extracts (Fig. 4, A and B, and fig. S8). In control chromatids (Δmock), both CAP-G (condensin I) and CAP-H2 (condensin II) localized to central axes, although CAP-H2 was confined to more internal regions than CAP-G. In nucleosome-depleted chromatids (ΔAsf1), CAP-G was somewhat diffuse, although CAP-H2 was still confined to central axes. In the absence of condensin I, although nucleosome-depleted chromatids (Δcond I–ΔAsf1) were more expanded than nucleosome-containing chromatids (Δcond I), nucleosomes had little impact on CAP-H2's distribution. In contrast, Asf1 depletion had a big impact on the assembly of chromatids in the absence of condensin II: Except for major satellite DNA regions, individual chromatids were no longer recognizable by DAPI or CAP-G signals (Δcond II–ΔAsf1), although single depletion of condensin II displayed only a subtle defect in chromatid assembly (Δcond II). These results suggested that condensin I requires nucleosomes to assemble fully compacted chromatids in the absence of condensin II. Although condensin II's ability to assemble chromatid axes was largely independent of nucleosomes (Fig. 4C), condensin II's rather cryptic function to support proper actions of condensin I becomes prominent under the nucleosome-depleted condition.

One of the most unexpected findings reported here is that nucleosome depletion resulted in assembly of chromatid structures with discrete axes, which were completely different from amorphous masses of chromatin observed upon depletion of condensins I and II. However, given the fact that condensins are evolutionarily older than histones (*6*) and that condensins and nucleosomes contribute to chromosome assembly at different levels, perhaps we should not be too surprised at this observation. Our current results confirm and extend the previous results that properly assembled nucleosomes allow productive action of condensin I (*11*) and also provide insight into functional collaboration between the two condensin complexes. An emerging idea is that condensin II first establishes axes with loosely organized loops and condensin I then facilitates lateral compaction of the nucleosomal loops. This idea is consistent with the spatiotemporally distinct behaviors of condensins I and II in vivo (*26*, *27*). It will be important in the future to address how the functional crosstalk between condensins and nucleosomes might build and organize large-scale chromosome structures at a mechanistic level.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/356/6344/1284/suppl/DC1
Materials and Methods
Figs. S1 to S8
Tables S1 and S2
References (*28*–*36*)

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Packaging without nucleosomes

The fundamental compaction of DNA in chromosomes is thought to start with the wrapping of DNA around nucleosomes, but is nucleosome wrapping required for the condensation of mitotic chromosomes? Shintomi *et al.* combined *Xenopus* egg extracts and mouse sperm nuclei and found that chromosome-like structures could be assembled in the near-complete absence of nucleosomes (see the Perspective by Kakui and Uhlmann). These "nucleosome-depleted" chromosomes were composed of condensin-enriched discrete axes and poorly organized chromatin loops. This finding challenges the textbook view of mitotic chromosome organization.

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