Mitotic chromatin condensation in vitro using somatic cell extracts and nuclei with variable levels of endogenous topoisomerase II.

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We report the development of a new method for producing mitotic extracts from tissue culture cells. These extracts reproducibly promote the condensation of chromatin in vitro when incubated with purified interphase nuclei. This condensation reaction is not species specific, since nuclei from chicken, human, and hamster cell lines all undergo chromatin condensation upon incubation with the extract. We have used this extract to investigate the role of DNA topoisomerase II (topo II) in the chromosome condensation process. Chromatin condensation does not require the presence of soluble topo II in the mitotic extract. However, the extent of formation of discrete chromosome-like structures correlates with the level of endogenous topo II present in the interphase nuclei. Our results further suggest that chromatin condensation in this extract may involve two processes: chromatin compaction and resolution into discrete chromosomes.

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We review micromechanical experiments on mitotic chromosomes. We focus on work where chromosomes were extracted from prometaphase amphibian cells, and then studied by micromanipulation and microfluidic biochemical techniques. These experiments reveal that chromosomes have well-behaved elastic response over a fivefold range of stretching, with an elastic modulus similar to that of a loosely tethered polymer network. More generally our results suggest a strategy for the use of micromanipulation methods for the study of chromosome structure. Keywords. Chromosome condensation is one of the major chromatin-remodeling events that occur during cell division. The changes in chromatin compaction and higher-order structure organization are essential requisites for ensuring a faithful transmission of the replicated genome to daughter cells. Condensin complexes can encircle DNA and also promote its supercoiling in vitro, but how these activities help them to orchestrate the changes in chromatin architecture is not known. Condensin II is nuclear during interphase, and has an important role in prophase chromosome formation following its activation by phosphorylation. Condensin I acts both in prophase and prometaphase in further compacting the chromosomes.