

Publication

A somatic cell-derived system for studying both early and late mitotic events in vitro

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We describe a cell-free system for studying mitotic reorganization of nuclear structure. The system utilizes soluble extracts prepared from metaphase-arrested somatic chicken cells and supports both the disassembly and subsequent partial reassembly of exogenous nuclei. By fluorescence microscopy, biochemical fractionation, protein phosphorylation assays and electron microscopy, we show that chicken embryonic nuclei incubated in extracts prepared from metaphase-arrested chicken hepatoma cells undergo nuclear envelope breakdown, lamina depolymerization and chromatin condensation. These prophase-like events are strictly dependent on ATP and do not occur when nuclei are incubated in interphase extracts. Compared to interphase extracts, metaphase extracts show increased kinase activities toward a number of nuclear protein substrates, including lamins and histone H1; moreover, they specifically contain four soluble phosphoproteins of Mr 38,000, 75,000, 95,000 and 165,000. Following disassembly of exogenous nuclei in metaphase extracts, telophase-like reassembly of a nuclear lamina and re-formation of nuclear membranes around condensed chromatin can be induced by depletion of ATP from the extract. We anticipate that this reversible cell-free system will contribute to the identification and characterization of factors involved in regulatory and mechanistic aspects of mitosis.

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