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RUVs Drive Chromosome Decondensation after Mitosis

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Condensation of chromosomes during mitosis is required for their segregation into daughter cells but must be reversed to allow for postmitotic functions. In this issue of *Developmental Cell*, Magalska et al. (2014) show that the ATPases RuvBL1/2 drive postmitotic chromatin decondensation, demonstrating that this is an active process.

Cell division requires wholesale reorganization of cellular contents, including the chromosomes, which condense to enable their accurate distribution by the mitotic spindle. The dramatic events of mitosis end abruptly when chromosomes decondense and resume their interphase functions. Although much is known about chromosome condensation, the molecules and events driving the return to a decondensed interphase state are still poorly understood (Vagnarelli, 2012). In this issue of *Developmental Cell*, Magalska et al. (2014) recapitulate this process in *Xenopus* egg extracts and show that postmitotic chromosome decondensation requires a distinct set of molecular factors, including RuvB-like members of the AAA+ ATPase family.

A number of factors have been reported to be essential for establishing and maintaining the characteristic, condensed X-shaped structure of mitotic chromosomes (Figure 1). Among them are topoisomerase IIα, condensins, and the chromokinesin KIF4A (Vagnarelli, 2012), all of which are enzymes that hydrolyze ATP, illustrating the energetic requirement for chromosome compaction and resolution. The process also requires activation of several mitotic kinases including Cdk1/cyclin B, Mps1, Aurora B, and Haspin, which appear to function at least in part by initiating changes in histone tail modifications that alter nucleosome interactions and thereby permit higher-order packing of chromatin fibers (Kagami et al., 2014; Wilkins et al., 2014; Vagnarelli, 2012).

Because mitotic exit is driven by inactivation of mitotic kinases accompanied by activation of PP1 and PP2A phosphatases (Wurzenberger and Gerlich, 2011), a straightforward model of chromosome decondensation might be that condensation-promoting activities are simply reversed, allowing chromosomes to relax into their interphase state. For example, the PP1 phosphatase appears to prime chromatin for decondensation by removing mitosis-specific histone modifications from mitotic chromatin (Vagnarelli et al., 2011), and the Aurora B kinase, which phosphorylates histone tails to alter nucleosome interactions and promote chomatin fiber packing, is thought to be extracted from chromosomes by the p97 AAA+ ATPase (Ramadan et al., 2007). Now, Magalska and colleagues significantly shift our perspective by showing that in addition to the undoing of mitotic events, postmitotic chromosome decondensation also depends on its own set of specific factors in an active process that requires both ATP and GTP (Figure 1).

The key to the authors' success was adaptation of the *Xenopus laevis* cytoplasmic egg extract system to study postmitotic changes in chromosome structure. Over the years, *Xenopus* egg extracts together with sperm nuclei have been used to reconstitute and study numerous cellular processes, including many aspects of chromosome biology such as replication, condensation, cohesion, and decondensation (Maresca and Heald, 2006). However, until now decondensation has mostly been studied in the specialized context of sperm chromatin, which is packed tightly with protamines and whose decondensation requires nucleoplasmin, a chaperone that is not expressed in somatic cells (Philpott and Leno, 1992). Magalska et al. used mitotic chromatin isolated from HeLa cells and, perhaps not surprisingly, discovered that sperm decondensation and mitotic chromosome decondensation have different molecular requirements. The authors took advantage of the highly synchronized cell-cycle state of the egg extract, which can easily be converted from mitosis to interphase, as well as the fact that it can be prepared in milliliter volumes, to employ a fractionation and purification strategy. Using this elegant, activity-based approach, Magalska et al. isolated two factors essential for postmitotic chromosome decondensation: RuvBL1 and RuvBL2 (also known as pontin and reptin; Nano and Houry, 2013).

RuvB-like proteins are highly conserved members of the AAA+ superfamily of ATPases, which form a characteristic hexameric ring. In some contexts, RuvBL1 and RuvBL2 have been shown to act as a heterododecameric complex, and in others they have been shown to exhibit antagonistic activities. Functionally, RuvB-like proteins have been linked to diverse cellular processes, including telomerase assembly, small nucleolar ribonucleoprotein (snoRNP) assembly, chromatin remodeling, transcription, spindle assembly, DNA damage repair, apoptosis, and several signaling pathways (Nano and Houry, 2013). Magalska et al. (2014) now add chromatin decondensation to this list. Interestingly, in this context, RuvBL1 and RuvBL2 act redundantly but are not required for the subsequent steps of nuclear envelope reformation and nuclear pore assembly, indicating a chromatin-specific role. The authors show the RuvBL1/2 both localize to postmitotic decondensing chromatin. Inhibition or depletion of RuvBL1/2 impaired chromatin decondensation, and this could be rescued by addition of recombinant RuvBL1/2 but not ATPase-deficient mutants. Depletion

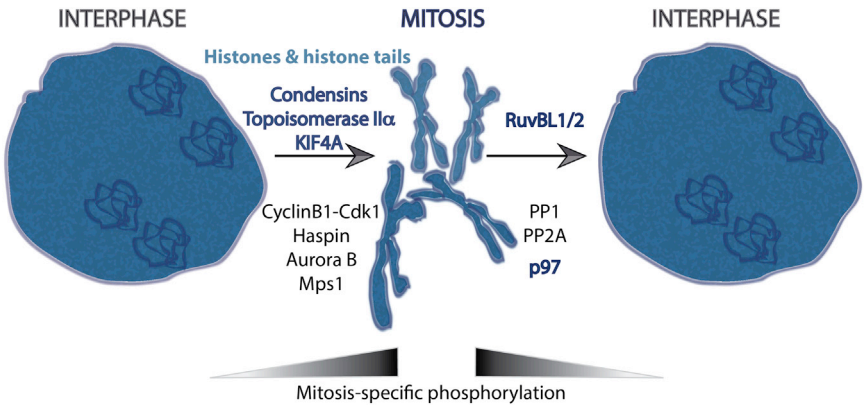

Figure 1. Chromatin Decondensation at Mitotic Exit Is Not Just a Reversal of Condensation
Interphase chromosomes are decondensed but occupy distinct territories within the nucleus. Chromosome condensation requires a number of factors that hydrolyze ATP (dark blue). Condensins I and II can generate positive supercoils in DNA, while topoisomerase IIα resolves entanglements, and the function of chromokinesin KIF4A is unclear. These factors act on chromatin, whose component histones and their posttranslational modifications are considered essential for establishing condensation state (light blue), and this is regulated by mitotic kinases such as Cyclin B1/Cdk1, Aurora B, and Haspin. Mps 1 regulates condensin. Chromosome decondensation is triggered at mitotic exit as mitotic kinase activity drops, PP1 and PP2A dephosphorylate substrates, and p97 contributes to inactivation of Aurora B. In addition, the activity of RuvBL1/2 plays a key role in establishing the decondensed state of postmitotic chromatin.

of endogenous nucleotides and use of non-hydrolyzable analogs revealed that chromatin decondensation requires ATP and GTP. Notably, RuvBL1/2 could not support mitotic chromatin decondensation in the absence of egg extract, suggesting a requirement for additional cofactors.

The study from Magalska et al. (2014) raises several questions. We still do not understand mechanistically what RuvB-like proteins are doing. What are their cofactors and substrates, and what is the role of ATP hydrolysis? RuvBL1/2 proteins may act directly to decompact chromatin, or they could act indirectly by extracting other factors, analogous to how the AAA+ ATPase p97 removes Aurora B from chromosomes at mitotic exit. Interestingly, Magalska et al. (2014) showed that RuvBL1/2 were necessary for decondensation but not sufficient for it to occur. In addition to fractions containing RuvBL1/2 proteins, the inclusion of an additional fraction was required during successive purification steps to reconstitute chromosome decondensation activity. What is the composition of this fraction, and does it contain molecular factors essential to prime chromatin decondensation, or cofactors for RuvBL1/2? What aspect of the process requires GTP? This study provides not only a physiological system to address these questions but also a molecular handle on the process, which sets the stage for detailed molecular investigation of postmitotic chromosome decondensation.

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