Regulation of chromosome condensation and sister chromatid separation

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To ensure faithful transmission of genetic information through mitosis and meiosis, the DNA is replicated during interphase to generate a pair of sisters that remain linked together by a molecular 'glue' to ensure proper alignment on the mitotic spindle. The chromosomes condense and line up on the mitotic spindle, then the two sisters separate and migrate to the opposite poles, followed by cell cleavage yielding identical daughter cells. The data generated in the recent years have provided meaningful insights in understanding chromosome condensation[1,2], sister chromatid separation[3,4], and elucidation of large-scale chromosome organization[5]. The salient features relating to organized chromosome segregation deciphered recently are highlighted here.

Chromosome condensation

The DNA of eukaryotic chromosomes must be elaborately folded to fit within the confines of the nucleus. As such, the replicated DNA strands are systematically compacted during interphase through fundamental process of chromosomal condensation – for each chromosome this means packing of about 4 cm of DNA into a rod 10 μm long, and 1 μm in diameter[6]. The degree of folding changes locally through chromatin remodelling to allow specific transcription of individual genes and globally to allow chromosome segregation during cell cycle. As cells enter mitosis, chromosome condensation during prometaphase resolves the bulk of each chromatid's chromatin from that of its sister[7]. Early attempts to elucidate the process of condensation suggested that there are major changes in the phosphorylation of histones as cells enter mitosis[8], and one protein kinase that performs this phosphorylation (the complex of Cdc2 and cyclin B) is the principal biochemical activity that induces mitosis[9]. Despite this correlation, however, the importance of histone phosphorylation in mitotic chromosome condensation remained unclear. Using *Xenopus* egg extract as the experimental system that lacks transcription activity, it has now been shown that a five-subunit protein complex dubbed as 'condensin' is essential for mitotic chromosome condensation[1]. Condensin converts interphase chromatin into mitotic-like chromosomes by reconfiguring DNA by introducing an ordered global positive writhe in the presence of topoisomerase I and adenosine triphosphate[2]. Such knotting requires ATP hydrolysis and cell cycle-specific phosphorylation of condensin. Comparison of the condensin complex from interphase and mitotic extracts reveals that three of its subunits become phosphorylated in the mitotic extract and that only the mitotic form of the complex has the ability to supercoil DNA. Cdc2 is likely to be the kinase that phosphorylates and activates condensin that may trigger mitotic chromosome condensation; and depletion of the former would enable decondensation[1,6]. Based on specific study on mitotic chromosomes assembled *in vitro*, it is estimated that there is one unit of condensin for 5–10 kb of DNA[2]. Kimura *et al.*[2] further suggest that positive solenoidal supercoiling is a mitosis-specific strategy for chromatin organization.

Sister chromatid separation

One of the most dramatic events of the eukaryotic cell cycle is the separation of sister chromatids at the metaphase-to-anaphase transition, that otherwise remain paired along the entire length till their attachment to the mitotic spindle. It has long been suspected that destruction of sister chromatid cohesion, rather than a major change in traction exerted by the spindle, is responsible for sudden separation of sister chromatids at the metaphase-to-anaphase transition. Cohesion between sisters resists the pulling forces exerted by microtubules attached to sister kinetochores[10] and thereby ensures that sister chromatids attach to microtubules emanating from opposite spindle poles[11].

There are important clues as to the molecular nature of the cohesive structures that hold sisters together and the mechanism by which it is suddenly broken at the onset of anaphase[12]. Cohesion between sister chromatids is established during DNA replication, and depends on a multisubunit protein complex called 'cohesin'[3,13]. In the budding yeast, *Saccharomyces cerevisiae*, cohesin is comprised of at least four subunits: Scc1, Scc3, Smc1 and Smc3 (refs 13, 14). A similar cohesin complex has been implicated in sister chromatid cohesion in *Xenopus* extracts[7]. The SMC (structural maintenance of chromosomes) proteins were originally identified in yeast as key elements of chromosome segregation and have since been recognized in a wide range of organisms[15]. Attachment of sister kinetochores to the mitotic spindle during mitosis generates forces that would immediately split sister chromatids were it not opposed by cohesion. Cohesion is essential for the alignment of chromosomes in metaphase but must be abolished for sister separation to start during anaphase. Uhlman *et al.*[3] have demonstrated that in the budding yeast sister chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. The loss of sister chromatid cohesion in fact depends on a separating protein (separin) called Esp1. Using a mutant Scc1 that is resistant to Esp1-dependent cleavage and which blocks both sister chromatid separation and the dissociation of Scc1 from chromosomes, it has been shown that Esp1 causes dissociation of Scc1 from chromosomes when sister chromatids separate[3]. Esp1 may therefore have a direct role in removing Scc1 from chromosomes by stimulating its cleavage by proteolysis. The evolutionary conservation of separins indicates that the proteolytic cleavage of cohesion proteins might be a general mechanism for triggering anaphase.

Is cleavage of cohesin a cause or consequence of sister separation? With the development of methods to locate specific sequences in fixed nuclei by *in situ* hybridization and localization of fluorescent binding proteins, it became possible to identify proteins – called Pds1 in budding yeast that had to be destroyed by the 'anaphase-promoting

complex' to enable the sisters to separate[16,17]. So, two different kinds of proteolysis are needed to initiate sister separation. The first is activation of the anaphase-promoting complex, the enzyme which leads to the wholesome destruction of Pds1. This, in turn, frees Esp1 to introduce two surgical snips in Scc1, thereby destroying the cohesin complex[17]. A similar functional situation has also been observed for vertebrate sister chromatid separation as well, where a protein called 'securin' which is analogous to Pds1 in budding yeast and Cut 2 in fission yeast has been identified[4]. An analysis of related data[17] indicates that changes in the cohesin subunits are responsible for the differences between chromosome segregation in mitosis and meiosis, and a single change in chromosomal protein may be enough to cause the altered pattern of chromosome segregation that is responsible for sexual reproduction.

Further, besides identification of proteins involved in sister chromatid cohesion, efforts have also been made to identify DNA elements involved in the process. Using a budding yeast minichromosome centromere assay, Megee and Koshland[18] were able to identify a centromeric element *CDEIII* that was necessary (but not sufficient) for cohesion, suggesting that the centromere cassette contains DNA elements that mediate sister chromatid cohesion, although, there may be another DNA element outside the cassette that mediates cohesion. Their data, however, do emphasize that at least in budding yeast, cohesion and kinetochore activities are coordinated through a common sequence element. Their observations further suggested that cohesion factors may bind to chromosomes nonspecifically like histones or specifically to multiple sites. In either case, the DNA elements are functionally redundant.

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OPINION

From Auschwitz to Indian science

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A World Energy Assessment meeting in Cracow (Poland) a few days ago gave me an opportunity to visit the concentration camps at Auschwitz and Birkenau 50 km away. Brought from all over Nazi-occupied Europe during World War II, about 1.5 million innocent victims, overwhelmingly Jews, either went directly to the gas chambers and the crematoria at Auschwitz and Birkenau, or indirectly via the camps where they were held prisoners until they were too weak to labour.

The tour of the camps, now preserved as a museum, created a completely unexpected feeling. The scale was so enormous that it is difficult, particularly because the camps have been unpopulated since 1944, to imagine that there used to be human beings here. Human belongings – toothbrushes, shoes and suitcases – were piled separately from floor to ceiling in huge rooms, but the aggregate was more reminiscent of factory inputs. Even the enormous mound of human hair was raw material for the manufacture of tailor's lining cloth. If Auschwitz was unbelievable, its neighbour Birkenau located 3 km away, beggared the imagination. Birkenau was spread over 175 hectares with 300 buildings each capable of housing 1000 inmates. It was a scale-up from the pilot plant demo at Auschwitz with a peak of 20,000 prisoners to full scale commercialization at Birkenau with 100,000 prisoners in August 1944. The powerful impression that persisted was of detailed engineering resulting in '... the immense technological complex created ... for the purpose of killing human beings' (*Auschwitz – How Many Perished*, Yad Vashem Studies, Jerusalem, 1991, vol. XXI,