Chromosome condensation: **Packaging the genome**

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The packaging of centimetre long DNA molecules into compact metaphase chromosomes is essential for genome segregation in anaphase. The chromosomal condensin complex plays a crucial part in this packaging, and important new insight into condensin action *in vitro* and *in vivo* has recently been gained.

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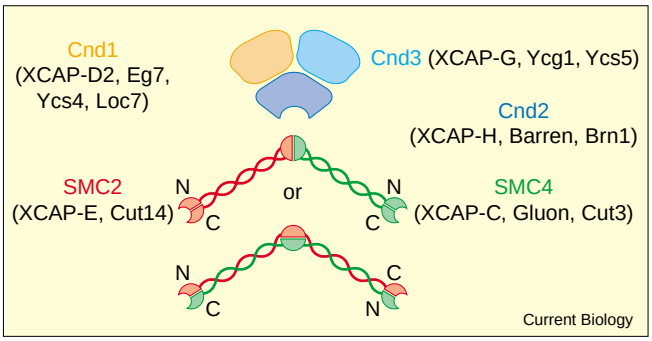
A total of 4 metres of DNA are packed into human chromosomes at each mitosis, and it is clear that elaborate mechanisms are at work to organise multiple levels of condensation of the DNA [1]. It is therefore of great interest to identify the proteins involved in this process. One successful approach in the past was to isolate condensed metaphase chromosomes and to ask what proteins they contain [2,3]. Apart from histones, the two most abundant components that were found on metaphase chromosomes were topoisomerase II and the so called 'condensin' complex. If two strands of DNA get entangled during the condensation process, topoisomerase II ensures their resolution by transiently opening and re-sealing of one of the strands. Without the activity of topoisomerase II, entangled DNA would cause chromosome breaks in mitosis. As it is difficult to repair DNA breaks during mitosis, cells might have taken precaution to avoid any such break by supplying a high density of topoisomerase II distributed all along the chromosome.

Figure 1



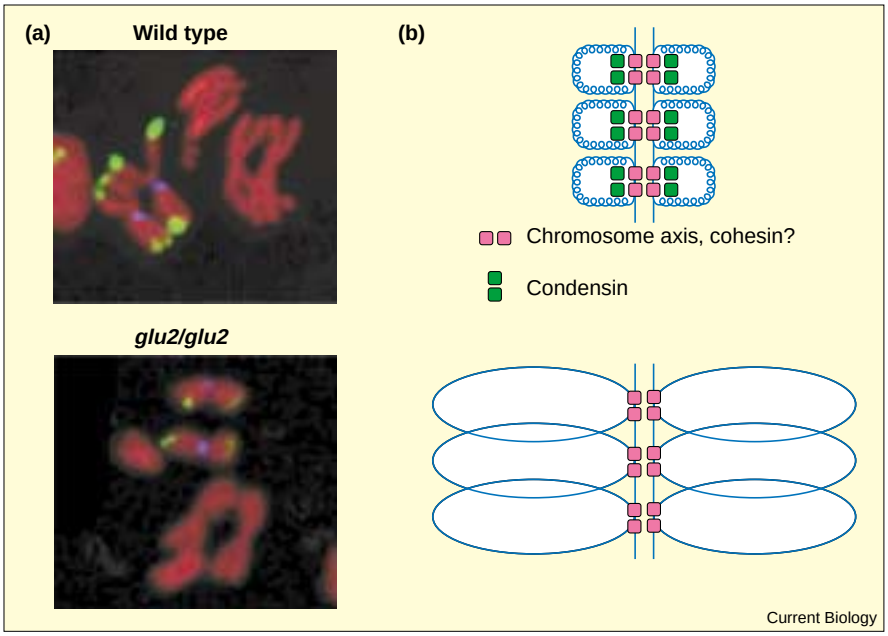
A model for the architecture of the condensin complex. A unified nomenclature is suggested for all subunits based on the names in use for budding yeast subunits Smc2 and Smc4, and fission yeast subunits Cnd1–Cnd3. Names in use for homologous subunits in other organisms are given in parenthesis. Cnd2 might link the regulatory subcomplex (Cnd1–Cnd3) with the core subcomplex (SMC2 and SMC4) [10]. Two possible arrangements of the SMC subunits within the core subcomplex are depicted.

The other highly abundant component found on mitotic chromosomes is the condensin complex. Condensin has been isolated from *Xenopus* egg extracts as a five subunit protein complex, and in a *Xenopus in vitro* chromosome assembly assay the condensin complex is essential for the formation of condensed chromosomes [4]. The binding of many molecules of the condensin complex to DNA *in vitro* in the presence of ATP introduces global positive writhe, and can produce positive supercoils [5,6]. This suggests a function of condensin as organiser of the topology of DNA loops in chromosomes. The condensin complex is well conserved, and a similar condensin complex from human cells can replace *Xenopus* condensin in the *Xenopus* chromosome assembly assay [7].

Condensin can be divided into two subcomplexes. A core complex consists of two 'structural maintenance of chromosome' (SMC) subunits, SMC2 and SMC4. When first isolated, these subunits were called *Xenopus* chromosome associated proteins, XCAP-C and XCAP-E; the nomenclature of condensin subunits is currently very diverse, so I suggest unified names in Figure 1. SMC proteins form V-shaped dimers with two long arms of anti-parallel coiled coil connected by a flexible hinge (Figure 1). At both ends of the arms, the amino and carboxyl termini of the proteins join to form head domains. Whether the coiled coils in this heterodimer form within each SMC subunit and the dimer interface is at the hinge, or whether the coiled coils form between the two subunits and the dimer interface extends along the entire molecule, remains to be seen (Figure 1).

The SMC core complex can bind to naked DNA and shows ATPase activity. Both DNA binding and ATPase are weaker than in the complete condensin complex, and the core complex alone is not able to reconfigure DNA. Such dimeric SMC complexes are also found in bacteria, and the crystal structure of the head domain of *Thermotoga maritima* SMC has now confirmed that the amino and carboxyl termini of the SMC molecules together form a head structure that belongs to the 'ATP binding cassette' (ABC) family of ATPases [8]. The crystal structure of the DNA repair protein Rad50, which also forms extended coiled coil dimers with SMC-like heads, shows how ATP-binding might regulate higher-order interactions between SMC heads, and how the head–head interactions might influence their mode of DNA binding [9].

Figure 2



(a) Examples of mitotic chromosomes in *Drosophila* neuroblasts of *glu2/glu2* (*smc4*) mutant larvae as compared to wild type larvae. DNA is in red, centromeres of chromosome 2 are marked blue, and telomeres of chromosome 2 are marked green by *in situ* hybridisation. The distance in length from centromere to telomere is unaltered between wild type and *glu2* mutant chromosomes, however compaction and resolution of sister chromatid axes is defective. (Reproduced from [17].) **(b)** A model explaining the observations in (a). A role of condensin specifically in compacting chromosome loops is proposed, while the organisation of the chromosome axis is performed by different proteins, maybe involving cohesin [14].

The consequences of such regulated interactions for the biology of the condensin complex remain to be explored.

The other three subunits, Cnd1–Cnd3 — the *Xenopus* versions of which are called XCAP-D2, XCAP-H and XCAP-G (Figure 1) — together form another subcomplex, called the regulatory subcomplex [10]. Antibodies against the Cnd2 subunit prevent association of the regulatory complex with the core complex, suggesting the interaction between the two is mediated via Cnd2. The regulatory subcomplex does not seem to have intrinsic catalytic properties, but its association with the SMC core complex increases the latter's DNA binding and ATPase activity. The regulatory subcomplex is also required for the binding of condensin to DNA packed into nucleosomes or into chromatin, and for all its activities in reconfiguring DNA and promoting chromosome condensation *in vitro*. It will be most interesting to see how the regulatory complex fits onto and influences the three-dimensional structure of the SMC core complex.

The SMC2 component of the condensin complex has also been isolated by genetic means in budding yeast, as the product of a gene required for chromosome condensation and segregation [11]. In both budding and fission yeast, an essential five-subunit condensin complex, homologous to *Xenopus* condensin, has meanwhile been characterised [12,13]. In yeast strains mutant for condensin subunits, chromosomes fail to condense and the segregation of chromosomes in anaphase remains incomplete, leading to chromosome loss. Why do condensation defects lead to a failure in chromosome segregation? During condensation, chromosomes gain shape and the two sister chromatid axes separate from each other and from other chromosomes. This is a prerequisite for efficient chromosome segregation in anaphase. In budding yeast, cohesin, a distinct but structurally similar protein complex to condensin, is also required for full chromosome condensation [14,15], indicating that there is some cross-talk between sister chromatid cohesion and condensation.

What have we learned about the cellular function of condensin in higher eukaryotes? The *Drosophila barren* gene encodes the homolog of Cnd2, and consistent with the expected function of condensin, mutation of *barren* was found to cause incomplete chromosome segregation in anaphase [16]. The *gluon* gene encodes the *Drosophila* homolog of SMC4, and the effects of *gluon* mutation have recently been analysed [17]. In *gluon* mutant cells, mitotic chromosomes appeared diffuse with poorly discernible sister chromatid axes, indicative of condensation defects. Again, anaphase bridges were frequently observed, and consequently various chromosomal rearrangements and aneuploidy.

An interesting finding was made when the metaphase chromosomes of *gluon* mutant cells were examined more closely. Although the chromosome arms were diffuse and obviously not well condensed, their length was the same as fully condensed wild-type chromosomes [17] (Figure 2).

This indicates that condensin, at least in *Drosophila*, is not essential to form a shortened chromosome axis, but is required to condense the loops of chromatin that extend from this axis. If condensin is not responsible for organising the condensed chromosome axis, what is? Is it cohesin, as has been suggested in yeast [14]? Cohesin is dispensable for chromosome assembly and condensation in a *Xenopus in vitro* system [18], but it will be interesting to compare these observations with what happens in *Drosophila* cohesin mutants.

How do cells regulate the binding of condensin to chromosomes and its condensation activity? *Xenopus* condensin must be phosphorylated by Cdc2 kinase in mitosis to become active for condensation [19]. In fission yeast, condensin is excluded from the nucleus until it becomes phosphorylated by Cdc2 kinase [12]. The phosphorylation of histone H3, which is often used as a marker for chromatin condensation during mitosis, coincides with the binding of condensin [20,21]. This prompted the hypothesis that condensin recognises and binds to phosphorylated histone H3. However, *Xenopus* condensin seems in principle able to bind to chromatin in the absence of H3 phosphorylation [10]. In *Drosophila*, the aurora B kinase is required for histone H3 phosphorylation, as well as for the binding of condensin to chromosomes [21]. Together, these observations suggest that the phosphorylation of histone H3 and the binding of condensin to chromosomes are independent, but regulated by a common pathway. The human A-kinase anchoring protein AKAP95 was also shown to be required for condensin binding to chromosomes [22]. Future work is necessary to analyse how these factors together regulate chromosome condensation during mitosis.

When the localisation of *Drosophila* condensin was analysed in interphase cells, the SMC4 subunit and Cnd2/Barren were not seen to colocalise [17]. Could this indicate the existence of separate core and regulatory complexes of condensin *in vivo*? On fully condensed chromosomes in mitosis, condensin was not found globally covering the condensed DNA, as might have been expected from the non-processive way of condensin action *in vitro*. Condensin was found confined to a striking corkscrew-like pattern along the axes of chromosome arms. Any implication of this pattern for condensin function remains to be explored. And as in yeast [13], *Drosophila* condensin was found particularly concentrated along ribosomal DNA and at the heterochromatic centromere regions.

Is there more to the relationship between heterochromatin and condensin? This may well be the case, as indicated by a recent high-resolution mapping experiment in *Drosophila* [23], which showed that condensin colocalises with Polycomb group proteins to Polycomb response elements. These are chromosomal regions that act to transcriptionally repress neighbouring domains by initiating their packaging into heterochromatin-like structures. Indeed, the condensin subunit Cnd2/Barren was found to be required to maintain the *Fab-7* silent region, and a *barren* mutation led to developmental misregulation of this locus. But not only is condensin required at Polycomb response elements for transcriptional silencing, Polycomb group proteins in turn seem to be also required for general chromosome condensation during mitosis. Mutation of the Polycomb group protein Polyhomeotic was found to cause chromosome condensation and segregation defects very reminiscent of the condensin mutant phenotype [23]. This indicates that condensation by condensin might be inseparable from the general regulation of chromosomal topology outside of mitosis, as well as during mitosis. Consistent with this idea, human condensin subunits were found not only on chromosomes during mitosis, but also at discrete spots in interphase nuclei [20].

Will we understand chromosome condensation once we fully understand how the condensin complex works? Certainly not. It is predicted that there are a number of factors in addition to condensin that are essential for chromosome condensation and have the ability to bind to the tails of histones, but the identities of these putative factors are not yet known [24]. We are just at the beginning of the challenging, but very exciting, endeavour to learn how cells arrange and package their most precious molecule, DNA.

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