Condensin in Chromatid Cohesion and Segregation

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Abstract

After replication of genomic DNA during the S phase, 2 chromatids hold together longitudinally. When cells enter mitosis, the paired sister chromatids start to condense and then segregate into individual chromatids except for the centromeric region. Upon attachment of microtubules to the kinetochores, subsequent pulling of the 2 sister chromatids by the spindles towards opposite poles results in 2 completely separated chromatids. Besides more than 100 kinds of kinetochore proteins, several key proteins such as cohesin, separase, shugoshin, and condensin contribute to chromatid cohesion and segregation. Among these proteins, condensin, a protein complex composed of 5 subunits discovered 2 decades ago, has been extensively studied in terms of the maintenance of chromosome morphology as its major function. Recent studies on condensin uncovered its role in chromatid cohesion and segregation, which will be reviewed in this article.

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Condensin Architecture

Condensin was first discovered as a condensation factor of mitotic chromosomes by Hirano and Mitchison [1994] through a chromosome reconstitution experiment using *Xenopus* oocyte extract. Condensin is composed of 2 SMC (structure maintenance of chromosome) and 3 non-SMC subunits. While yeast has only 1 condensin complex (*Schizosaccharomyces pombe*: Cut14 and Cut3 for SMC subunits; Cnd1, Cnd3, and Cnd2 for non-SMC subunits; *Saccharomyces cerevisiae*: Smc2 and Smc4 for SMC subunits; Ycs4, Ycg1, and Brn1 for non-SMC subunits), in higher eukaryotes, 2 types of condensin complexes are known, condensin I and II. Both complexes are composed of 2 SMC subunits, Smc2/CAP-E (homologue of Cut14 or Smc2) and Smc4/CAP-C (homologue of Cut3 or Smc4), which are common for condensin I and II, and 3 non-SMC subunits, CAP-D2 (homologue of Cnd1 or Ycs4), CAP-G (homologue of Cnd3 or Ycg1), and CAP-H (homologue of Cnd2 or Brn1) for condensin I and CAP-D3, -G2, and -H2 for condensin II. The SMC subunits have a globular hinge domain and a head domain with long coiled-coils connecting the 2 domains (fig. 1).

Fig. 1. Molecular architectures of condensin I (left) and condensin II (right). In eukaryotes, Smc2 and Smc4 form a heterodimer which interacts with 3 non-SMC subunits to form the 5-subunit complex. Counterpart of condensin in yeast is condensin I in higher eukaryotes.

The SMC subunits (Smc2 and Smc4) form a heterodimer through the interaction of the 2 subunits at the hinge domain, whereas each head domain of the 2 subunits has ATPase activity, which is required for the introduction of supercoiling to DNA. Recent studies have suggested that non-SMC subunits expressing HEAT-repeat sequences (CAP-D2 and -G for condensin I and CAP-D3 and -G2 for condensin II) mediate the recruitment of condensins onto chromosomes [Piazza et al., 2014]. As for its structural contribution, condensin is well known as one of the major components of the chromosome scaffold and is required for the maintenance of mitotic chromosome architecture [Ono et al., 2003]. Our previous proteome analysis of human metaphase chromosomes indicated that the molar ratio of hCAP-C to 100 molecules of histone H4 is 0.32 at the most, corresponding to condensin binding to the chromatin fiber every 33 kb on average [Uchiyama et al., 2005]. Our recent study using super-resolution microscopy revealed that the chromosome scaffold appears at the axis of the sister chromatids, which was first discovered by Paulson and Laemmli [1977] and was later identified as barber pole model [Maeshima and Laemmli, 2003], and is constructed in a twisted double-stranded fashion in each chromatid [Poonperm et al., 2015] (fig. 2).

Fig. 2. Model of double-stranded chromosome scaffold based on super-resolution microscopy.

Three-Dimensional Structure of Condensin

It has been debated whether the hinge portion of condensin SMC takes an open conformation, giving 2 separated coiled-coils, while the inner side of the hinge is exposed for DNA recognition. Anderson et al. [2002] reported the structural difference between condensin SMC and cohesin SMC heterodimers based on electron microscopy observations where condensin SMC takes a closed conformation while cohesin SMC has an open conformation. The crystal structures of the prokaryotic SMC hinge and of the SMC hinge from *Thermotoga maritima* with short coiled-coils were reported [Haering et al., 2002]. The structure revealed that the hinge domain is dimerized by the formation of β -sheets at the 2 binding surfaces of monomers with a donut-shaped, ring-like overall structure. The 2 coiled-coils were extended to opposite directions, corresponding to the open conformation (fig. 3, upper left). Among eukaryotes, the crystal structures of mouse condensin hinge and cohesin hinge have been independently reported. These structures revealed that the eukaryotic SMC hinges form similar folds as the prokaryotic and bacterial SMC hinge domains, demonstrating that the overall structure of the SMC hinge domain is universal for a wide variety of SMC proteins,

Fig. 3. Three-dimensional structures of the SMC hinge dimers with coiled-coils. A certain length of coiled-coils is necessary to take a rod-like conformation as observed in bacterial (*Pyrococcus furiosus*) and eukaryotic (*Saccharomyces cerevisiae* and *Homo sapiens*) SMC hinge dimers with coiled-coils.

although the configuration of condensin coiled-coils still remained elusive. Recent crystal structure analyses of the budding yeast condensin Smc2/Smc4 hinge with long coiled-coils (about 110 amino acids) and the human condensin Smc2/Smc4 hinge with middle coiled-coils (about 30 amino acids) allowed to answer this lasting question; the 2 coiled-coils protruding from each subunit have the same orientation, indicating the formation of a rod-shape structure (fig. 3, right panels) [Soh et al., 2015; Uchiyama et al., 2015]. The direct interaction between the 2 coiled-coils was confirmed by hydrogen/deuterium exchange mass spectrometry (HDX-MS), showing that the rod-shaped structure (not open, but closed conformation) is maintained in solution. In addition, the homodimer of the prokaryotic SMC hinge with long coiled-coils had a rod conformation as the Smc2/Smc4 hinge with coiled-coils from yeast and human (fig. 3) [Soh et al., 2015]. This

study suggested that the *T. maritima* SMC dimer failed to maintain the rod conformation due to the insufficient length of the coiled-coil segment. In addition, HDX-MS showed that the interaction between the Smc2/Smc4 hinge with middle coiled-coils and single-stranded DNA (ssDNA) is mediated through the outer surface of the SMC hinge portion. The signature of structural alterations across the hinge-hinge dimerization interface upon ssDNA binding proposed the presence of a half-opened state of the SMC hinge heterodimer [Uchiyama et al., 2015]. Such structural change potentially relates to the DNA loading mechanism of condensin, in which the hinge domain functions as an entrance gate as previously proposed for cohesin. This is, however, not the case for condensins based on the fact that the coiled-coils are still interacting with each other, even when DNA binding induces structural changes in the hinge region.

Condensin Is Concentrated at the Centromere or Kinetochore

Related to centromere or kinetochore, the accumulation of *S. pombe* condensin at mitotic kinetochores was previously reported [Nakazawa et al., 2008]. In vertebrate cells, enrichment of hCAP-E in the centromeric regions of mitotic chromosomes, which contain large amounts of repetitive DNAs, was observed [Poonperm et al., 2015], and condensin II is enriched in the kinetochores during mitosis as its phosphorylation level increases in an aurora B and/or hMis6/CENP-I-dependent manner [Ono et al., 2004; Nakazawa et al., 2008]. Enrichment of condensin in pericentromeric regions was also reported in a number of organisms including budding yeast [Eckert et al., 2007; D'Ambrosio et al., 2008; Kim et al., 2013]. As centromere-binding protein mutants show unequal chromosome segregation [Nabeshima et al., 1998; Hayashi et al., 2004], condensin mutants in yeast also exhibited decreased fidelity of sister kinetochore segregation, whereas the bulk of the chromosomal DNA remained associated [Nakazawa et al., 2008]. The functional implication for the accumulation of condensin around the centromere region is thus to ensure faithful segregation under appropriately applied forces in concert with another SMC-containing protein complex, cohesin. Post-translational modifications of condensin subunits regulate their function in vivo. Casein kinase 2 (CK2)-dependent phosphorylation of condensin increases during interphase and decreases on chromosomes during mitosis [Takemoto et al., 2006]. In contrast, a positive regulatory mode of condensin I is induced by mitosis-specific Cdc2-mediated phosphorylation [Kimura et al., 1998; Takemoto et al., 2004]. Studies on the phosphoproteome and proteome in mitotic cells by quantitative mass spectrometry-based proteomics revealed that dephosphorylation of CAP-G by protein phosphatase 6 is opposed to CK2-dependent phosphorylation and required for the proper chromosome condensation and segregation in anaphase [Rusin et al., 2015]. Recently, based on chromatin immunoprecipitation sequencing experiments, Sutani et al. [2015] reported that fission yeast condensin is localized to centromeres, ribosomal DNA (rDNA), and unwound DNA on the chromosome arms in a transcription-dependent manner in mitosis. Importantly, in their analysis, human condensin I was shown to be bound to unwound DNA at the transcription start sites. They also reported that binding of condensin to unwound DNA restores ssDNA produced during transcription to double-stranded DNA (dsDNA), and that this elimination of unwound DNA is a prerequi-

site for introducing global positive supercoiling into DNA, thereby contributing to chromosome condensation. These results are consistent with the previous findings that condensin preferentially binds to ssDNA rather than dsDNA in vitro and also that condensin has a role in the reannealing process of ssDNA to dsDNA [Sutani and Yanagida, 1997; Hirano and Hirano, 2002; Sakai et al., 2003; Akai et al., 2011].

Functional Relationship between Condensin and Cohesin

Cohesin, also containing SMC protein complexes (Smc1 and Smc3 in human), is considered to entrap the sister chromatids after replication, followed by the removal from the chromatid arms by proteolytic degradation via prophase pathways [Waizenegger et al., 2000; Losada et al., 2002], although the exact localization of cohesin in interphase has rarely been confirmed by direct microscopic observation. However, a small fraction of cohesin remains on chromosomes at the centromere region, maintaining centromeric cohesion until anaphase. Two mechanistic force balances between condensin and cohesin have been proposed. Ono et al. [2013] suggested that the distance between sister chromatids is determined by the balance between resolving forces exerted by condensin II and cohesive forces by cohesin along the 2 chromatids. Besides the centromeric region, condensin and cohesin are accumulated at rDNA, and condensin plays a fundamental role in rDNA segregation [D'Amours et al., 2004]. Condensin binds to tRNA gene transcription complexes, leading to the clustering of tRNA genes [Haeusler et al., 2008]. In this case, condensin does not simply contribute to the resolving of chromatin fibers. Altogether it can be concluded that the interplay between condensin and cohesin is essential for proper chromatin organization and successive segregation where cohesin mainly bundles chromatin fibers while condensin adjusts the shape of organized chromatin fibers.

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