Chromosome Dynamics Laboratory
Chief Scientist: Tatsuya Hirano (Ph.D.)

(0) Research field

CPR Subcommittee: Biology

Keywords: Chromosomes; Cell cycle; Mitosis; Condensins; SMC proteins

(1) Research background and long-term goals

The chromosome is at the heart of all activities in life. How does the large structure composed of DNA and proteins assembled, duplicated, and transmitted from generation to generation? This question is not only fundamental to basic biology but also relevant to our understanding of cancer cell proliferation and germ cell formation, thereby having broad clinical implications. Our laboratory is interested in understanding the molecular mechanisms of higher-order chromosome architecture and dynamics, with a major focus on a class of large protein complexes, known as condensins, which we discovered two decades ago. We take multidisciplinary approaches toward this goal, including biochemistry, structural biology, cell biology, and genetics. We are also interested in the evolution of chromosome dynamics and human diseases accompanying chromosome anomalies.

(2) Research activities (FY2021)

(A) Functional analyses of condensins I and II by using recombinant complexes

Many eukaryotic species have two different condensin complexes (condensins I and II), each of which is composed of five subunits. They share a pair of SMC (structural maintenance of chromosomes) core subunits but contain different sets of non-SMC regulatory subunits. It remains poorly understood mechanistically how condensins I and II drive mitotic chromosome assembly and how their cooperative actions are regulated during the cell cycle. To address these fundamental questions, we had established an *in vitro* functional assay using frog egg extracts, in which endogenous condensin complexes are replaced with recombinant (either wild-type or mutant) complexes. In FY2021, substantial progress was made in our understanding of the mechanism and regulation of condensin II. We found that the CAP-G2 subunit has a very strong negative impact on condensin II loading and condensin II-mediated chromosome axis assembly. The carboxyl-terminal tail region of CAP-D3 also contributes to the negative regulation of condensin II, possibly through physical interaction with CAP-G2. These observations start to uncover the question of to what extent condensins I and II are similar to and different from each other, in terms of both their molecular mechanisms and cell cycle regulation.

(B) Further development of chromosome assembly assays using frog egg extracts

Frog egg cell-free extracts provide a powerful experimental system in which mitotic chromosomes can be assembled in a test tube starting from simple substrates such as frog sperm nuclei or mouse sperm nuclei. In FY2021, we modified this system by introducing interphase nuclei derived from frog erythrocytes as a substrate for chromosome assembly. When sperm nuclei are used as substrates, nucleosome assembly and higher-order reorganization of chromatin occur simultaneously. In contrast, erythrocyte nuclei already contain fully assembled nucleosomes before being incubated with the extracts. Owing to the differences in the initial compositions, we found that sperm nuclei and erythrocyte nuclei impose differential requirements for chromatin remodelers and linker histones during the process of mitotic chromosome assembly. It is anticipated that this new experimental system will be useful to further enhance our understanding of how condensins and topoisomerase II functionally crosstalk with nucleosome assembly and dynamics.

(C) Role of condensin II in the transition from mitosis to interphase

Condensins I and II are subject to differential spatiotemporal regulation during the cell cycle. At the end of mitosis (i.e., telophase), for instance, condensin I dissociating from chromosomes is exported out of the assembling nucleus, whereas condensin II stays within the nucleus thereafter throughout interphase. The function of condensin II during interphase (especially at the transition from mitotic telophase to G1 phase) remains unknown. We had previously established a human cell line in which one of the condensin II-specific subunits can be degraded rapidly utilizing the auxin-inducible degron (AID) system and found that depletion of condensin II suppresses intranuclear dispersion of centromeres that occurs from telophase to early G1. In FY2021, we found that post-mitotic centromere dispersion occurs normally in cells depleted of cohesin. Interestingly, however, the suppression of centromere dispersion observed in the absence of condensin II is rescued when cohesin is depleted simultaneously. These observations shed light on the question of how cohesin takes over the architectural role from condensin II and establishes the global organization of interphase chromosomes.

(3) Members (FY2021)

(Chief Scientist)
Tatsuya Hirano

(Senior Research Scientist)
Takao Ono, Kazuhisa Kinoshita, Keishi Shintomi

(Postdoctoral Researcher)
Shoji Tane, Makoto Kozai, Toshiharu Fujita

(Technical Staff)
Yuki Masahara, Yuuki Aizawa

(Assistant)
Tomoko Maruyama

(Part-time Worker)
Miki Ebihara

(4) Representative research achievements (FY2021)

1. "A loop extrusion-independent mechanism contributes to condensin I-mediated chromosome shaping", Kinoshita K, Tsubota Y, Tane S, Aizawa Y, Sakata R, Takeuchi K, Shintomi K, Nishiyama T, Hirano T. J. Cell Biol. 221:e202109016 (2022).
2. "Guiding functions of the C-terminal domain of topoisomerase IIα advance mitotic chromosome assembly", Shintomi K, Hirano T. *Nat Commun.* 12:2917 (2021).
3. "Modality of mitotic chromosomes", Hirano T, The 44th Annual Meeting of the Molecular Biology Society of Japan (December 2, 2021, Virtual).
4. "DNA topology in mitotic chromosome assembly and shaping", Hirano T, The EMBO Workshop on "*DNA topology in genomic transactions*", (September 20-23, 2021, Virtual).

Laboratory Homepage

https://www.riken.jp/en/research/labs/chief/chromosome_dyn/index.html
http://www2.riken.jp/chromdyna/index_en.html