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 (FY2023)

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

Many eukaryotic species have two distinct condensin complexes (condensin I and condensin 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 have established an in vitro functional assay using frog egg extracts, in which endogenous condensin complexes are replaced by recombinant (either wild-type or mutant) complexes. In FY2023, we found that the CAP-D3 subunit of condensin II possesses a CAP-G2-interacting domain (termed the “HEAT docker”) located between its HEAT repeat core and the intrinsically disordered D3 C-tail. When the HEAT docker was deleted along the D3 C-tail, the resulting mutant condensin II complex displayed a higher chromosome loading activity than the complex lacking the D3 C-tail alone, leading to the formation of mitotic chromosomes similar to those produced by the wild-type condensin I complex. Thus, the manipulation of condensin II allowed us to convert it into a complex with condensin I-like activities.

(B) Single-molecule analysis of the coordinated actions of condensin I and topoisomerase II

We have previously shown that mitotic chromosomes can be reconstituted by mixing a simple substrate (frog sperm nuclei) and six purified proteins. Of these, two proteins, condensin I and topoisomerase II, are ATPases that can actively reconfigure DNA and chromatin structures. To understand how the two ATPases work together in the process of mitotic chromosome assembly, we asked what happens when topoisomerase II is added to a condensin I-mediated loop extrusion assay on single DNA molecules (in collaboration with Tomoko Nishiyama’s group at Kyoto University). By 2022, we found that, in the presence of a low concentration of topoisomerase II, a DNA loop formed by the action of condensin I is converted into a more compact structure (“lump”). In 2023, we extended this observation and found that DNA knots are formed in the lump, depending on the catalytic activities of condensin I and topoisomerase II. Interestingly, the C-terminal domain of topoisomerase II, which is not essential for its catalytic activity, is also required for the formation of the lump and the DNA knots within it. These results shed new light on the long-debated role of topoisomerase II in mitotic chromosome assembly.

(C) Toward a full understanding of the phosphoregulation of condensin I and condensin II

Mitotic chromosome assembly is thought to be under the control of the mitotic protein kinase cyclinB-Cdk1. Indeed, we have previously shown that Cdk1 phosphorylation activates the DNA supercoiling and chromosome reconstitution activities of condensin I. However, these demonstrations were made using both condensin I and cyclin B-Cdk1 purified from native sources. To dissect the phosphoregulation of condensin I more precisely, it is essential to develop an experimental system in which the native protein complexes are replaced by recombinant ones. In 2023, we successfully established a protocol in which recombinant cyclin B-Cdk1, together with its adaptor protein Suc1, efficiently phosphorylates the subunits of condensins I and II. We now plan to uncover the whole molecular picture of condensin phosphoregulation using multiple functional assays, such as chromosome reconstitution and DNA loop extrusion.

(D) <u>Collaboration of condensin II and cohesin in maintaining G2 phase chromosome structure</u>

Condensins I and II are subject to different spatiotemporal regulation during the cell cycle. For example, at the end of mitosis, for example, condensin I dissociates from chromosomes and is exported from the assembling nucleus, whereas condensin II stays in the nucleus thereafter throughout interphase. By FY2002, we found that, when cohesin was depleted from G2-arrested cells, local chromatin structures (~1 Mb scale) were affected, but large-scale chromosome territories were not. Similarly, when condensin II was depleted from G2-arrested cells, intermediate chromosome structures (~20 Mb scale) were compromised while chromosome territories appeared to be intact. In FY2023, we observed severe defects in the maintenance of chromosome territories in G2-arrested cells depleted of both cohesin and condensin II: in many cases, territories were deformed and localized to the periphery of the nucleolus. These observations suggest that cohesin and condensin II, although operating at different scales, cooperate to maintain the large-scale chromosome territories in G2 phase.

(3) Members (FY2023)

(Chief Scientist)
Tatsuya Hirano
(Senior Research Scientist)
Takao Ono, Kazuhisa Kinoshita, Keishi Shintomi
(Postdoctoral Researcher)
Makoto Kozai, Toshiharu Fujita
(Technical Staff)
Yuki Masahara, Yuuki Aizawa
(Assistant)
Tomoko Maruyama
(Part-time Worker)
Miki Ebihara

(4) Representative research achievements (FY2023)

1. Shintomi K, Masahara-Negishi Y, Shima M, Tane S, and Hirano T. “Recombinant cyclin B-Cdk1-Suc1 capable of multi-site mitotic phosphorylation in vitro”, PLoS One 19:e0299003 (2024).
2. Yoshida MM, Kinoshita K, Shintomi K, Aizawa Y, and Hirano T. “Regulation of condensin II by self-suppression and release mechanisms”, Mol. Biol. Cell, 35(2):ar21 (2024).
3. Yamamoto T, Kinoshita K, and Hirano, T. “Elasticity control of entangled chromosomes: crosstalk between condensin complexes and nucleosomes”, Biophys J. 122:P3869-3881 (2023).
4. Hirano T. “Condensins and mitotic genome folding”, Research Lecture at Nobel Forum, Karolinska Institutet (February 15, 2024, Stockholm, Sweden).
5. Hirano, T. “A tale of two condensins: molecular dissection of mitotic genome folding machines”, Gordon Research Conference on “Chromosome Dynamics” (June 25-30, 2023, Lucca, Italy).

Laboratory Homepage
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