Chromosome Dynamics Laboratory

Chief Scientist: Tatsuya Hirano (Ph.D.)

(0) Research fields

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 (FY2022)

(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. By FY2021, we obtained evidence that the N-terminal tail of the CAP-H subunit (H N-tail) is involved in the regulation of condensin I loading on chromatin during the process of mitotic chromosome assembly. In FY2022, we found that the mutant condensin I complex lacking the H N-tail is able not only to bind to chromatin but also to assemble mitotic chromosome-like structures even in interphase extracts, although wild-type condensin I cannot bind to chromatin at all under the same conditions. In addition, the mutant complex displayed higher DNA-binding activities in two “extract-free” in vitro assays. We also obtained evidence that phosphorylation of the C-terminal tail of the CAP-D3 subunit (D3 C-tail) regulates condensin II loading on chromatin and condensin II-mediated chromosome assembly. Based on these results, similarities and differences between condensin I and condensin II in their ability to assemble mitotic chromosomes are now beginning to be uncovered.

(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). 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, which we termed a “lump”. The same concentration of topoisomerase II alone did not induce any structural changes in the DNA. This preliminary result provided an important clue to our understanding of the coordinated actions of condensin I and topoisomerase II in mitotic

chromosome assembly.

(C) <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. At the end of mitosis (i.e., telophase), for example, condensin I dissociates from chromosomes and is exported out of the assembling nucleus, whereas condensin II stays in the nucleus thereafter throughout interphase. By FY2021, we found that a functional "handover" from condensin II to cohesin plays an important role in the establishment of G1 phase chromosome structure. In FY2022, we began to investigate the role of condensin II and cohesin in the maintenance of G2 phase chromosome structure. To this end, we developed experimental protocols to visualize specific chromosomes and specific chromosome loci by fluorescence in situ hybridization (FISH) and found that cohesin and condensin II are involved in the maintenance of local chromatin structures (~ 1 Mb scale) and intermediate chromosome structures (~20 Mb scale), respectively.

(3) Members (FY2022)

(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 (FY2022)

1. Sun M, Amiri H, Tong AB, Shintomi K, Hirano T, Bustamante C, and Heald R. "Monitoring the compaction of single DNA molecules in Xenopus egg extract in real time", Proc. Natl. Acad. Sci. USA. 120:e2221309120 (2023).
2. Tane S, Shintomi K, Kinoshita K, Tsubota Y, Yoshida MM, Nishiyama T, and Hirano T. "Cell cycle-specific loading of condensin I is regulated by the N-terminal tail of its kleisin subunit", eLife 11:e84694 (2022).
3. Yoshida MM, Kinoshita K, Aizawa Y, Tane S, Yamashita D, Shintomi K, and Hirano, T. "Molecular dissection of condensin II-mediated chromosome assembly using in vitro assays", eLife 11:e78984 (2022).
4. Yamazaki H, Takagi M, Kosako H, Hirano T, and Yoshimura SH. "Cell cycle-specific phase separation regulated by protein charge blockiness", Nat. Cell Biol. 24:625-632. (2022).
5. Hirano T. "Condensins: what we know and what we don't know", (Keynote speaker), Biochemical Society Meeting on "Genome organization by SMC complexes" (September 27-30, 2022, Edinburgh, UK).

Laboratory Homepage
https://www.riken.jp/en/research/labs/chief/chromosome_dyn/index.html
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