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, that 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 (FY2019)

(A) Functional analyses of condensins I and II in *Xenopus* egg extracts

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 *Xenopus* egg extracts, in which endogenous condensin complexes are replaced with recombinant (either wild-type or mutant) complexes. In FY2019, we took advantage of this assay to examine the function of intrinsically disordered regions (IDRs) present in the three regulatory subunits of condensin I, and found that at least two of them make unique contributions to fine-tuning the core activity of condensin I. It was also found that one of the regulatory subunits of condensin II have a negative role in targeting the complex onto chromosomes. These observations suggest that condensins I and II are equipped with intrinsic mechanisms that regulate their own activities in a highly elaborate manner.

(B) Refinement of an *in vitro* chromosome reconstitution assay using purified proteins

We had shown previously that structures resembling mitotic chromosomes can be assembled *in vitro* by incubating *Xenopus* sperm nuclei with only six purified proteins (i.e., core histones, three kinds of histone chaperones, topoisomerase II and condensin I). Although this experimental system had provided a powerful starting point for studying the molecular mechanism of mitotic chromosome assembly, there remained large room for further technical improvements. In FY 2019, we noticed that the action of topoisomerase II is highly sensitive to buffer conditions in this system, and that a C-terminal IDR of topoisomerase II, which is not highly conserved among different species, is responsible for such sensitivity. Further experiments allowed us to demonstrate that dynamic tethering of topoisomerase II to chromosomes via its C-terminal IDR plays a crucial role in proper chromosome assembly. These observations contribute to further refinement of the experimental system and implicate a crucial role of intracellular environments in mitotic chromosome assembly.

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

Condensins I and II are subject to different 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 by means of the auxin-inducible degron (AID) system. In FY2019, we took advantage of this cell line and found that condensin II contributes to intranuclear dispersal of centromeres that occurs from telophase to early G1. This function turned out to be unique to condensin II and was not attributed to condensin I. These observations provide an important clue to our understanding of condensin II's function in organizing interphase chromosome architecture.

(3) Members (FY2019)

(Chief Scientist)
Tatsuya Hirano

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

(Postdoctral Researcher)
Shoji Tane, Makoto Kozai

(Technical Staff)
Masami Shima, Yuuki Aizawa

(Assistant)
Tomoko Maruyama

(Part-time Worker)
Seiko Moriyama, Miki Ebihara

(4) Representative research achievements (FY2019)

1. "Molecular dynamics simulations of condensin-mediated mitotic chromosome assembly", Sakai Y, Hirano T, Tachikawa M. Methods Mol. Biol. 2004:319-334 (2019).
2. "Structural basis of HEAT-kleisin interactions in the human condensin I complex", Hara K, Kinoshita K, Migita T, Murakami K, Shimizu K, Takeuchi K, Hirano T, Hashimoto H. EMBO Rep. 20:e47183 (2019).
3. "Condensin-based chromosome organization: new insights from in vitro assays", Hirano T (Symposium speaker), ASCB | EMBO 2019 meeting (Washington DC, USA, December 9, 2019).
4. "The two faces of condensin I", Hirano T, EMBO Workshop on "SMC proteins" (Vienna, Austria, September 10, 2019).
5. "Condensin-based chromosome organization: new insights from cell-free extracts", Hirano T, Gordon Research Conference on "Chromosome Dynamics" (Newly, Maine, USA, June 27, 2019).

Laboratory Homepage

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