様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　５ 年　 ６ 月　 ８ 日現在

機関番号：８２４０１
研究種目：若手研究
研究期間：2020～2022
課題番号：２０Ｋ１５７２３

研究課題名（和文）Molecular mechanism of condensin II in the regulation of mitotic chromosome assembly

研究課題名（英文）Molecular mechanism of condensin II in the regulation of mitotic chromosome assembly

研究代表者

香西　誠（ＹｏｓｈｉｄａＭａｋｏｔｏＭｉｃｈａｅｌ）（Yoshida, Makoto）

国立研究開発法人理化学研究所・開拓研究本部・協力研究員

研究者番号：２０７９１４５３

交付決定額（研究期間全体）：（直接経費）　　3,300,000円

研究成果の概要（和文）：有糸分裂期に起こる染色体構築の変化は細胞分裂を成功させるために不可欠なプロセスである。コンデンシン複合体（コンデンシンIとII）は染色体構築の変化と構造維持において重要な役割を果たすタンパク質複合体である。分裂期染色体の構築におけるコンデンシンIのサブユニットの機能が明らかになりつつあるが、コンデンシンIIのサブユニットについての理解はいまだ乏しい。コンデンシンIIは5つのタンパク質（サブユニット）を持ち、機能解析を行うため、複合体を発現し、精製に成功した。様々の変異体を調べ、それぞれのサブユニットの機能を解明した。そして、コンデンシンIとの違いを示した。

研究成果の学術的意義や社会的意義
Mis-regulation of chromosome organization, primarily mediated by condensin protein complexes, can lead to the development of diseases and cancer. This study elucidates the mechanism of condensin II protein complex to understand how cell division is achieved without errors in chromosome organization.

研究成果の概要（英文）：Changes in chromosomal structure that occur during mitosis are essential for successful cell division. Condensin I and II are protein complexes that play important roles in compacting and maintaining chromosome structures. Although the function of condensin I subunits in the assembly of mitotic chromosomes is becoming clear, condensin II subunits remain poorly understood. To elucidate the function of condensin II and its five subunits, the complex was expressed and successfully purified. By testing various mutant complexes, the study was able to elucidate the functional roles of each subunit as well as the similarities and differences between condensin I and II.

研究分野：Molecular biology

キーワード：condensin　chromosome　mitosis　HEAT　phosphorylation

１．研究開始当初の背景

(1) Formation of mitotic chromosomes is an indispensable cellular process during cell division. The compaction of chromosomal DNA for vertebrate chromosome assembly is primarily mediated by two pentameric complexes: condensin I and condensin II.

(2) While both condensin complexes share the same pair of SMC (structural maintenance of chromosomes) ATPase subunits, SMC2 and SMC4, they differ by their non-SMC subunits. Condensin I contains CAP-D2, CAP-G, and CAP-H while condensin II contains CAP-D3, CAP-G2, and CAP-H2.

(3) Their componential differences provide unique roles for the spatiotemporally dependent process of assembling rod-shaped mitotic chromosomes, with condensin II initiating chromosomal compaction before condensin I. Mis-regulation of these processes often lead to genomic instability and aneuploidy, and can further lead to the development of cancer and diseases.

(4) While extensive studies started to unravel the molecular action of condensin I, the mechanistic functions of condensin II have remained largely unexplored.

２．研究の目的

(1) The purpose is to determine to what extent the action of condensin II differs from that of condensin I in the process of chromosome assembly. The functional roles of condensin II subunits will be elucidated as well as how they are regulated.

(2) A secondary purpose is to determine how condensin I and II collaborate with each other, both spatially and temporally, in the process of chromosome assembly.

３．研究の方法

(1) To determine the functional roles of condensin II, DNA constructs of the five mammalian subunits (mouse SMC4, mouse SMC2, human CAP-D3, human CAP-G2, and human CAP-H2) are placed in bacmids and transfected into insect cells to express the five proteins to form a complex. Condensin II complexes are purified with Streptactin beads using a StrepII-tag at the C-terminal end of CAP-D3, and further purified using the AKTA column chromatography system with a HiTrap Q column. The purified condensin II complex is collected and concentrated to use for biochemical assays. Recombinant mutant complexes are prepared similarly. For complexes that do not contain CAP-D3, a CAP-H2 construct with a StrepII-tag at the N-terminal end is used in the first step of the purification with Streptactin beads. Condensin I is purified by using Glutathione Sepharose beads to capture the GST-tag on SMC4.

(2) *Xenopus* egg extract is used for biochemical experiments to check chromosome assembly by condensin II. Metaphase-arrested egg extract is prepared from *Xenopus laevis* frog eggs that are centrifuged to separate soluble and insoluble fractions. The soluble fraction contains the protein machinery necessary for mitotic chromosome assembly within the egg extract. Endogenous condensins are depleted from egg extract by immunodepletion using antibodies targeting *Xenopus* condensin subunits. Recombinant condensin complexes are added into the immunodepleted egg extract and mouse sperm nuclei is incubated in the egg extract. Assembled chromosomes are isolated and stained by immunofluorescence to observe localization of condensins.

(3) In vitro buffer-based assay are conducted to test specific condensin II functions using recombinant condensin II complexes.

４．研究成果

(1) Recombinant condensin II holocomplex was successfully purified and tested in *Xenopus* egg extract (Figure 1). While depletion of endogenous condensins reduced the compaction of mitotic rod-shaped chromosomes, the addition of recombinant condensin II holo(WT) could restore the condensin II-mediated chromosome morphology, named chenille-like chromosomes, that is originally formed by endogenous condensin II. The results

suggest that recombinant mammalian condensin II can functionally replace the endogenous condensin II in the *Xenopus* egg extract for condensin II-mediated mitotic chromosome assembly, validating the assay in investigating the functions of condensin II.

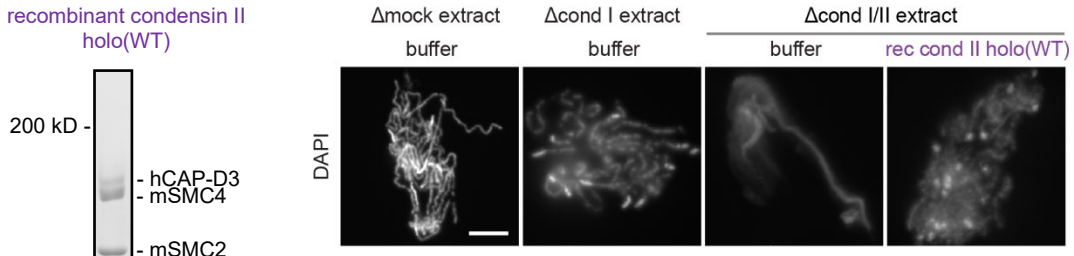

Figure 1. Recombinant mammalian condensin II can functionally replace endogenous condensin II in *Xenopus* egg extract.
Recombinant condensin II holo(WT) was added at 200 nM to *Xenopus* egg extract immunodepleted of both condensin I and II (ΔcondI/II) and tested alongside Δmock and ΔcondI extract and incubated with mouse sperm nuclei for 150 min before being fixed. DNA was counterstained with DAPI. Scale bar, 10 μm.

(2) Removal of CAP-D3 and CAP-G2 subunits from the condensin II complex resulted in a reduction in chromosome association by ΔD3(WT) and ΔD3ΔG2(WT) subcomplexes while ΔG2(WT) subcomplex overloaded on the chromosomal DNA. The results suggest that CAP-D3 is essential for chromosome assembly while CAP-G2 acts as a suppressor.

(3) Mutations in SMC2 and SMC4 ATPase domains revealed that condensin II requires ATPase activity for chromosome association and axis formation in chromosome assembly.

(4) Mutations in CAP-H2 central basic amino acid clusters 1 and 2 (BC1 and BC2) revealed that the BC1 and BC2 are important for chromosome association and axis formation in condensin II-mediated chromosome assembly.

(5) Deletion of the CAP-D3 C-terminal tail (C-tail) that is regulated by mitotic phosphorylation can form an enhanced condensin II holocomplex (holo[D3-dC]) that can overload onto chromosomal DNA. The results suggest that the C-tail acts as a suppressor.

(6) Thin chromosomes formed by holo(D3-dC) and ΔG2(WT) subcomplex are different, suggesting a chromosome bulk compaction function by CAP-G2 (Figure 2). Holo(D3-dC) makes more compacted, DAPI-enriched morphologies while ΔG(WT) makes thinner DAPI-reduced chenille-like chromosomes. However, combining the two deletion mutations (ΔG2[D3-dC]) forms morphologies similar to those formed by ΔG(WT), suggesting a partial overlap in their suppression mechanisms.

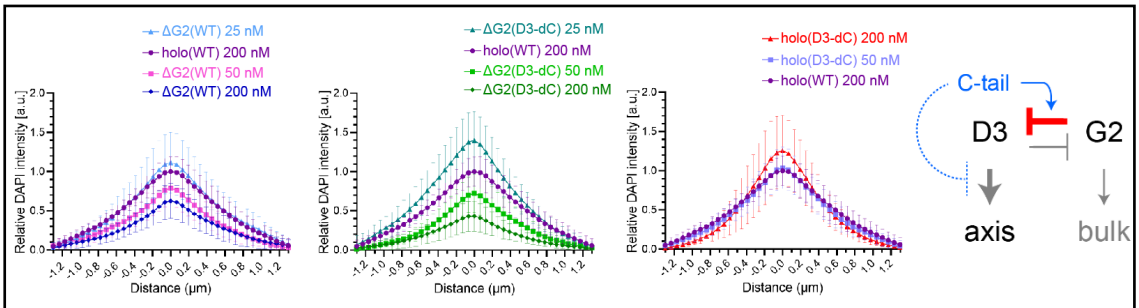

Figure 2. The CAP-D3 C-tail and CAP-G2 negatively regulate condensin II complex.
DAPI line scans were measured across the width of chromosomes formed by holo(WT), holo(D3-dC), ΔG2(WT), and ΔG2(D3-dC) that were added at 25, 50, and 200 nM to *Xenopus* egg extract. A model of regulatory roles of the CAP-D3 C-tail and CAP-G2 is proposed based on the results.

(7) Mutations at potential phosphorylation sites on the CAP-D3 C-tail reduce chromosome association and axis formation by condensin II, suggesting that condensin II is tightly regulated by phosphorylation at the CAP-D3 C-tail.

(8) In vitro chromatin binding assay using recombinant condensin II complexes and sperm nuclei recapitulates many of the results found using the egg extract assay. The results adds support to the findings from *Xenopus* egg extract assays in elucidating the functional roles of condensin II subunits.

(9) Chromosomes assembled by recombinant condensin I and II in *Xenopus* egg extract are dependent on the concentration and timing of the addition of recombinant complexes. Different concentrations of recombinant condensin I and II were tested, as well as the order of addition. Addition of recombinant condensin II to egg extract before condensin I addition helps form chromosomes with characteristics that are closer to those formed by endogenous condensins.

(10) This study is the first in determining the functional roles of condensin II non-SMC subunits in chromosome assembly. Furthermore, the results suggest similarities and differences between condensin I and II. The findings provide further insight on the molecular mechanism of action by condensin II. Further studies are necessary to fully elucidate how condensin II is regulated and how mis-regulation of condensin II can cause genome instability and the development of diseases.

＜引用文献＞

① Yoshida, M.M., Kinoshita, K., Aizawa, Y., Tane, S., Yamashita, D., Shintomi, K., & Hirano, T. (2022). Molecular dissection of condensin II-mediated chromosome assembly using in vitro assays. eLife, 11:e78984.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　2件）

１．著者名 Yoshida Makoto M、Kinoshita Kazuhisa、Aizawa Yuuki、Tane Shoji、Yamashita Daisuke、Shintomi Keishi、Hirano Tatsuya	４．巻 11
２．論文標題 Molecular dissection of condensin II-mediated chromosome assembly using in vitro assays	５．発行年 2022年
３．雑誌名 eLife	６．最初と最後の頁 ―
掲載論文のDOI（デジタルオブジェクト識別子） 10.7554/eLife.78984	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Tane Shoji、Shintomi Keishi、Kinoshita Kazuhisa、Tsubota Yuko、Yoshida Makoto M、Nishiyama Tomoko、Hirano Tatsuya	４．巻 11
２．論文標題 Cell cycle-specific loading of condensin I is regulated by the N-terminal tail of its kleisin subunit	５．発行年 2022年
３．雑誌名 eLife	６．最初と最後の頁 ―
掲載論文のDOI（デジタルオブジェクト識別子） 10.7554/eLife.84694	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計3件（うち招待講演　0件／うち国際学会　1件）

１．発表者名 Makoto Michael Yoshida
２．発表標題 Intrinsic subunit CAP-G2 negatively controls condensin II-mediated mitotic chromosome assembly
３．学会等名 第38回染色体ワークショップ・第19回核ダイナミクス研究会
４．発表年 2021年

１．発表者名 Makoto Michael Yoshida
２．発表標題 Regulation of condensin II-mediated chromosome assembly by a pair of intrinsic HEAT-repeat subunits
３．学会等名 第39回 染色体ワークショップ・第20回 核ダイナミクス研究会
４．発表年 2021年

１．発表者名 Makoto Michael Yoshida
２．発表標題 A self-suppression mechanism in condensin II helps to fine-tune mitotic chromosome assembly
３．学会等名 Genome organisation by SMC complexes（国際学会）
４．発表年 2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

―

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関