Analysis of the role of Aurora B on the chromosomal targeting of condensin I

Ai Takemoto1,2,3,4, Akiko Murayama1, Miyuki Katano1, Takeshi Urano5, Koichi Furukawa5, Shigeyuki Yokoyama3,4, Junn Yanagisawa1, Fumio Hanaoka2,6 and Keiji Kimura1,2,*

1Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba Science City, Ibaraki 305-8572, Japan, 2Cellular Physiology Laboratory, Discovery Research Institute, RIKEN and SORST, Japan Science and Technology Agency, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan, 3Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan, 4RIKEN Genomic Sciences Center, 1-7-22, Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan, 5Department of Biochemistry II, Nagoya University Graduate School of Medicine, Showa-ku, Nagoya, Japan and 6Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamada-oka, Suita, Osaka 565-0871, Japan

Received January 23, 2007; Revised March 1, 2007; Accepted March 1, 2007

ABSTRACT

During mitosis, chromosome condensation takes place, which entails the conversion of interphase chromatin into compacted mitotic chromosomes. Condensin I is a five-subunit protein complex that plays a central role in this process. Condensin I is targeted to chromosomes in a mitosis-specific manner, which is regulated by phosphorylation by mitotic kinases. Phosphorylation of histone H3 at serine 10 (Ser10) occurs during mitosis and its physiological role is a longstanding question. We examined the function of Aurora B, a kinase that phosphorylates Ser10, in the chromosomal binding of condensin I and mitotic chromosome condensation, using an in vitro system derived from Xenopus egg extract. Aurora B depletion from a mitotic egg extract resulted in the loss of H3 phosphorylation, accompanied with a 50% reduction of chromosomal targeting of condensin I. Alternatively, a portion of condensin I was bound to sperm chromatin, and chromosome-like structures were assembled when okadaic acid (OA) was supplemented in an interphase extract that lacks Cdc2 activity. However, chromosomal targeting of condensin I was abolished when Aurora B was depleted from the OA-treated interphase extract. From these results, it is suggested that Aurora B-dependent and Cdc2-independent pathways of the chromosomal targeting of condensin I are present.

INTRODUCTION

At the onset of mitosis, dispersed chromatin in the interphase nucleus is resolved and packaged into physically separate compact structures, called mitotic chromosomes. This step, mitotic chromosome condensation, is a prerequisite for the accurate segregation of chromosomes, the failure of which is considered to lead to aneuploidy, cancer and cell death. Despite its importance, the molecular mechanism underlying dynamic changes of the higher order chromosome structure is poorly understood for a long time (1,2).

However, considerable progress has been made in the identification of proteins implicated in this process, using yeast genetics or an in vitro system derived from Xenopus egg extract. For example, topoisomerase II and condensin(s) have been identified as essential factors required for mitotic chromosome condensation. Topoisomerase II is an enzyme that catalyzes the strand passage of double-stranded DNA (3), and its activity is required for the proper condensation of mitotic chromosomes, as demonstrated by yeast genetics and the Xenopus in vitro system. Condensin(s) plays a more direct role in mitotic chromosome condensation, and also in the maintenance of its structure. About a decade ago, 13S condensin (now referred to as condensin I) was identified using a Xenopus system (4,5). Condensin I is composed of two structural maintenance of chromosomes (SMC), SMC4/CAP-C and SMC2/CAP-E subunits and three other non-SMC (CAP-D2, -G and -H) subunits, all of which are conserved widely in eukaryotes, ranging from yeast to humans (6–9). Condensin I, purified from either Xenopus mitotic egg

*To whom correspondence should be addressed. Tel: +81-29-853-6632; Fax: +81-29-853-4605; Email: kekimura@sakura.cc.tsukuba.ac.jp

© 2007 The Author(s)
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/2.0/uk/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
extract or mitotic HeLa cells, possesses activity to induce positive superhelical tension in double-stranded DNA in an ATP-hydrolysis-dependent manner (10–12). This activity is stimulated by mitosis-specific phosphorylation and reduced by CK2-mediated interphase phosphorylation, which suggests that it may be a physiologically relevant activity required for mitotic chromosome condensation (13,14). More recently, a second condensin complex, condensin II, which possesses the same set of SMC subunits as condensin I but contains a different set of non-SMC subunits (CAP-D3, -G2 and -H2), was identified (15,16). The two complexes show distinct distributions on the mitotic chromosome axis and have distinct functions during mitotic chromosome condensation (15,17,18).

All eukaryotic chromosomes condensed during mitosis, and two mitotic kinases are implicated in the establishment and maintenance of chromosomes in a condensed state by regulating condensin complexes. One pathway is mediated by the master mitotic kinase, Cdc2. In Xenopus or humans, biochemical activity and the chromosomal association of condensin are stimulated by Cdc2 (4,13). In fission yeast, the phosphorylation of SMC4 by Cdc2 is required for nuclear localization of the condensin holo complex (19). The other pathway is thought to require Aurora B kinase (pPl1 in budding yeast); however, the role of Aurora B in the regulation of condensin has been less characterized. Aurora kinases are serine/threonine protein kinases that play critical roles in many aspects of cell division, including the centrosome cycle, spindle assembly, chromosome condensation, the spindle checkpoint and cytokinesis (20,21). In mammals, at least three Auroras (A, B and C) are present and they share similar structures, but differ in their expression patterns, subcellular localization and substrates. Among them, Aurora B phosphorylates histone H3 at its serine 10 (pSer10) residue during mitosis, which may be related to mitotic chromosome condensation. Aurora B forms a tight complex with inner centromere protein (INCENP) and survivin, and more recently, a fourth subunit of Aurora B complex was identified (22,23). It is also reported that Aurora C phosphorylates Ser 10 of nucleosomal H3 (24).

Formerly, Aurora B was believed to be implicated in the chromosomal localization of condensin I and mitotic chromosome condensation. Inhibition of Aurora B function results in the loss of H3 phosphorylation and the recruitment of condensin I to chromatin in S. pombe (25,26). Depletion of Aurora B by RNA interference (RNAi) blocks mitotic chromosome condensation and the targeting of condensin in Drosophila (27) and C. elegans (28,29); however, this notion is now challenged. For example, condensin I was normally targeted to mitotic chromosomes in the Aurora B-depleted Xenopus mitotic egg extract, in which Ser10 phosphorylation was almost abolished (30,31). Thus, different results were obtained among different species and different experimental systems, and the exact role of Aurora B in mitotic chromosome structures remains to be determined.

In this study, we focused on the regulation of the more abundant form of condensin, condensin I, by Aurora B, using cell-free extracts derived from Xenopus eggs. Chromosomal targeting of condensin I was decreased to about 50% by the depletion of Aurora B from mitotic Xenopus egg extract. A substantial amount of condensin I was targeted to sperm chromatin in the okadaic acid (OA)-treated interphase extract that lacks Cdc2 activity, accompanied by H3 phosphorylation. Furthermore, partial chromosome condensation was introduced into the OA-treated interphase extract. The OA-dependent chromosomal targeting of condensin I, and H3 phosphorylation were almost abolished by the depletion of Aurora B from the extracts. Taken together, we conclude that a subpopulation of condensin I is bound to chromosomes in an Aurora B-dependent manner.

MATERIALS AND METHODS

Antibodies

Rabbit antisera were raised against synthetic peptides corresponding to the carboxy-terminal sequences of Xenopus Aurora A (KNSQLKKKDEPLPAQ), Aurora B (SRRLPPVYQSTQSK), INCENP (SNRHHLAVGYGLKY) and Cdc2 (KSSLPDNQIRN). An amino-terminal cysteine was added to each peptide for sulfhydryl coupling. Conjugation of the peptides to keyhole limpet hemocyanin and affinity purification of antibodies was performed as described (32). Affinity-purified antibodies were diluted to a concentration of 1 μg/ml, and used for immunoblotting. Two micrograms of each antibody were used for immunoprecipitation from 100 μl of Xenopus egg extracts, as described previously (4).

An antibody that recognizes the phosphorylated form of histone H3 at Ser10 (pSer10) was used. An antibody that recognizes the phosphorylated form of histone H3 at Ser10 (pSer10) was used.

Preparation of Xenopus egg extracts

Mitotic and interphase high-speed supernatants were prepared as described previously (4), and was used throughout the study. In some experiments, OA was supplemented into the interphase extract.

Immunodepletion

For immunodepletion of Aurora A from Xenopus egg extracts, 10 μg of affinity-purified anti-Aurora A antibody was incubated with 30 μl of Protein A-Sepharose beads for 1 h. To deplete Aurora B, a mixture containing 10 μg of anti-Aurora B and 10 μg of anti-INCNP was used. For Cdc2 depletion, 10 μg of anti-Cdc2 was used. After washing the antibody-coupled beads with XBE2 [10 mM K-Hepes (pH 7.7), 100 mM KCl, 2 mM MgCl2, 0.1 mM CaCl2, 5 mM K-EGTA (pH 7.7) and 1.7% sucrose], 100 μl of Xenopus egg extracts supplemented with the ATP-regenerating system (1 mM Mg-ATP, 10 mM creatine phosphate and 50 μg/ml creatine kinase) were mixed, and incubated on a rotating wheel at 4°C for 1 h. The supernatants were recovered and then incubated with a fresh batch of the same beads. After incubating for 1 h, the supernatant was recovered and used as a depleted extract.
Biochemical analysis of chromatin or chromosomes assembled in vitro

Sperm nuclei (5000 nuclei/μl) were incubated with high-speed supernatant supplemented with the ATP-regenerating system at 22 °C for 2 h. Samples were placed on ice for 10 min, and chromatin was isolated by centrifugation through a 30% sucrose cushion in XBE2 at 10,000 × g for 15 min. The chromatin fraction was subjected to SDS-PAGE followed by Coomassie staining or immunoblotting. In some experiments, the amounts of chromosome-bound condensin subunits in each extract were quantitated using the LAS 1000 imaging system (Fujifilm, Allendale, NJ, USA).

Immunofluorescence staining

Immunofluorescence staining of chromatin or chromosomes assembled in the extracts was performed as described with minor modifications (33,34). For fixation, 1% paraformaldehyde was used rather than 2%. For the detection of condensin I, 1 μg/ml affinity-purified anti-XCAP-H antibody was used.

Purification of Xenopus condensin I

Xenopus condensin I was purified from mitotic, interphase or 4 μM OA-supplemented interphase egg extracts using the anti-XCAP-G antibody coupled to protein A-Sepharose beads, as described previously (10).

RESULTS

Depletion of Aurora B from mitotic extract results in the reduction of chromosomal association of condensin I

Aurora is a family of serine/threonine protein kinases that regulates many aspects during cell division. Among them, Aurora B, which phosphorylates nucleosomal Ser10 during mitosis, has long been believed to be correlated with mitotic chromosome condensation and segregation; however, different results are obtained depending on the experimental systems. To re-evaluate the exact roles of Aurora B in chromosome condensation and chromosomal targeting of condensin complex, we used an in vitro system derived from Xenopus egg extract. There are two types of the Xenopus egg extracts; one is a high-speed supernatant in which chromosomes are assembled without preceding DNA replication, the other is a low-speed supernatant that allows one complete round of DNA replication before entry into mitosis. Chromatin assembly using the latter extracts may reflect more physiological process (35); however, we used a high-speed supernatant throughout this study because it is more simple system for analyzing the chromosomal loading of condensin I.

We immunodepleted Aurora B, Aurora A and Cdc2 from mitotic egg extracts, and examined Ser10 phosphorylation, chromosomal targeting of condensin I, and chromosome structures (Figure 1). The immunodepletion efficiency of each kinase was estimated to be more than 94% by quantitative immunoblotting (Figure 1A). First, the phosphorylation level of histone H3 at Ser10 residue was examined. Aurora A depletion barely affected the phosphorylation of Ser10 (Figure 1B, lanes 11–13). When Aurora B was depleted from mitotic extract, the phosphorylation level of Ser10 of the chromatin-bound H3 was greatly reduced to that in the interphase extract (Figure 1B, lower, lanes 8–10), while Aurora B depletion had little impact on the phosphorylation of Ser10 of H3 in the extracts (Figure 1B, upper, lanes 8–10). These results demonstrate that Aurora B is the major kinase phosphorylating Ser10 of nucleosomal H3, while other kinase(s) are also able to phosphorylate the soluble form of H3 at Ser10 in Xenopus egg extract. Cdc2 depletion resulted in a loss of Ser10 phosphorylation of chromatin-bound and soluble forms of H3 (Figure 1B, lanes 14–16), suggesting that Ser10 phosphorylation during mitosis is downstream of Cdc2. Next, we tested the chromosomal targeting of condensin I (Figure 1C). Sperm chromatin was incubated with each extract, isolated through a sucrose cushion, and the chromatin-associated condensin I was analyzed by immunoblotting using XCAP-E and XCAP-G. Condensin I was bound to sperm chromatin in a mitosis-specific manner, and the association was almost abolished by Cdc2 depletion (Figure 1C, lanes 14–16). Aurora B depletion resulted in an approx. 50% reduction of chromosomal targeting of condensin I subunits (Figure 1C, lanes 8–10). On the other hand, the chromosomal targeting of condensin I was barely affected by mock depletion or Aurora-A depletion (Figure 1C, lanes 5–7 and 11–13). Alternatively, chromatin-associated proteins were analyzed by Coomassie blue staining (Supplementary Figure 1A), and the intensity of the bands of XCAP-C, -E and -G was quantitated using the Las 1000 imaging system (Fujifilm) (Supplementary Figure 1B). This experiment also indicates that Aurora B depletion resulted in an about 50% reduction of chromosomal targeting of condensin I subunits (Supplementary Figure 1B, lanes 1, 7 and 11). However, it is questionable that the 50% reduction of chromosomal association of condensin I in the Aurora B-depleted extract is a direct effect of the loss of Aurora B. For example, it is possible that Aurora B-depletion results in the reduction of Cdc2 activity in the Aurora B-depleted mitotic extract, which may lead to decreasing the amount of chromosome-associated condensin I. To address the criticism, we compared the Cdc2 kinase activity between control and Aurora B depleted-mitotic egg extract by MPM2 blotting. But, no significant difference was observed (data not shown).

The assembled structures were then fixed and stained with Hoechst to test the effect of the depletion of each kinase on chromosome condensation (Figure 1D). Sperm chromatin was converted into a condensed chromosome-like structure in a mitotic extract (Figure 1Db), but no condensation occurred in the interphase (Figure 1Da) or Cdc2-depleted mitotic extract (Figure 1Dd). When Aurora B was depleted from mitotic extracts (Figure 1Dd), the chromosome structure was almost indistinguishable from undepleted (Figure 1Db) or mock-depleted mitotic extracts (Figure 1Dc). These results indicate that chromosome condensation is dependent on Cdc2, but occurs in the Aurora B-depleted mitotic extract, in which the
chromosomal association of condensin I is reduced and Ser10 phosphorylation is abolished.

OA treatment of interphase extract results in the partial chromosome association of condensin I

Figure 1 has shown that some populations of chromosomal targeting of condensin I were regulated by Aurora B; therefore, we next tested whether condensin I is able to target to chromosomes in the absence of Cdc2, when downstream kinases, such as Aurora B, are activated. It is known that the addition of OA, an inhibitor of type 1 and type 2A phosphatases, into interphase extracts promotes the activation of downstream mitotic kinases in the absence of Cdc2 activity. We therefore supplemented OA into the interphase egg extract and examined its effects (Figure 2). The phosphorylation of chromatin-associated H3 at Ser10 in the OA-treated interphase extract reached a similar level to that in the mitotic extract (Figure 2A). This result is consistent with the previous report, in which...
RNAi depletion of Glc7/PP1 resulted in the phosphorylation of Ser10 (36). Under this condition, a portion of condensin I subunits was targeted to the assembled chromatin: about 12.5% as much condensin I was associated with chromatin as in the mitotic extract (Figure 2B). Next, we examined the chromatin structure by Hoechst staining (Figure 2C, upper). When OA was supplemented into the interphase extract, sperm chromatin was converted into partially condensed individual chromatin fibers (Figure 2Cc), which are completely different from the round-shaped chromatin sphere in the interphase extract (Figure 2Cb). However, the chromatin fibers assembled in the OA-treated interphase extract were thinner and much more fragile than chromosomes assembled in a mitotic extract (Figure 2Ca). We also investigated the localization of condensin I by immunostaining using anti-XCAP-H antibody, because it is reported that condensin is bound to the specific regions of chromatin (Figure 3C, middle lower), such as rDNA and centromere (37,38), was regulated by Aurora B; however, only weak staining signal was observed on the chromosome-like structure assembled in the interphase extract supplemented with OA (Figure 2Cf). Moreover, no specific region was intensively stained even when the signal was enhanced by long exposure (Figure 2Ch).

Then, to compare the structural integrity of the chromosomes assembled in the OA-supplemented interphase extract with that assembled in the mitotic extract, the assembled structures were treated with buffers containing increasing concentrations of KCl (Figure 2D).

Figure 2. Stimulation of chromosomal binding of condensin I by OA in interphase extracts. (A) Sperm nuclei were incubated with mitotic extract (lanes 1–3), interphase extract (lanes 4–6), interphase extract supplemented with 1.2 (lanes 7–9), 3.6 (lanes 10–12) or 12 μM (lanes 13–15) of OA at 22°C for 2 h. Chromatin-bound proteins were dissolved with SDS-PAGE sample buffer, and 12.5% (lanes 1, 4, 7, 10 and 13), 25% (lanes 2, 5, 8, 11 and 14) and 50% (lanes 3, 6, 9, 12 and 15) of each sample were separated by SDS–PAGE, and immunoblotted with anti-phospho H3. (B) Samples were prepared as described in (A), and 6.25% (lane 1), 12.5% (lanes 2, 4, 7, 10 and 13), 25% (lanes 3, 5, 8, 11 and 14) and 50% (lanes 6, 9, 12 and 15) of samples were blotted using anti-XCAP-E (upper), and anti-XCAP-G (lower) antibodies. (C) Sperm chromatin was assembled in the mitotic extract (a, d), interphase extract (b, c, g), and interphase extract supplemented with 3.6 μM OA (c, f, h). Samples were fixed and stained with Hoechst (a, b, c; first low), and anti-XCAP-H antibody (d, e, f; second low, short exposure: g, h; third low, long exposure). Bar, 10 μm. (D) Sperm chromatin was assembled in the mitotic extract (a, c, e) or interphase extract supplemented with 3.6 μM OA (b, d, f). After assembly, the reaction mixtures were supplemented with the indicated extra concentration of KCl at 22°C for 20 min, fixed, and stained with Hoechst. Bar, 10 μm.
Salt treatment of the chromosomes assembled in the mitotic extract resulted in a more extended and loosened structure (Figure 2Dd and g); however, the structures of individual chromosome axes were still observed even when treated with buffer containing extra 300 mM KCl. On the other hand, the chromosome-like structures assembled in the OA-treated interphase extract were fragile and easily broken by salt treatment (Figure 2De and h), indicating that they were qualitatively distinct from rigid chromosomes assembled in the mitotic extract.

The next question is whether partial chromosome condensation in the OA-supplemented interphase extract requires condensin I: it is possible that other factors activated by OA were implicated in chromosome condensation (39). To test this possibility, OA was supplemented into condensin I-depleted *Xenopus* egg extracts, and the chromatin structure was fixed and stained with Hoechst (Figure 3). The efficiency of immunodepletion was examined by immunoblotting with the indicated antibodies (Figure 3A). Next, we examined the amount of chromatin associated condensin I. Condensin I was absent from chromatin fraction assembled in the condensin I-depleted extract with OA (Figure 3B, lane 9), in contrast to substantial amount of condensin I was attached to chromosomes assembled in the OA-treated interphase extract (Figure 3B, lane 7). Interestingly, chromosome condensation did not take place in the condensin I-depleted extract when OA was supplemented into the extract; only thin chromatin fibers were observed (Figure 3CF). Thus, it is likely that OA-dependent chromosome condensation depends on condensin I.

Aurora B is the kinase implicated in the partial association of condensin I and chromosome condensation

Many kinases are activated by the addition of OA in the absence of Cdc2. Therefore, the next issue is to identify the kinase(s) that induces Ser10 phosphorylation, partial chromosome association of condensin I, and partial chromosome condensation in the OA-treated interphase extract. It is also possible that residual Cdc2/cyclin B in the interphase extract may be activated by this treatment, thereby causing these events. To address these questions, OA is supplemented into the interphase extract that had been depleted of Aurora B, Aurora A or Cdc2 (Figure 4). The efficiency of immunodepletion was determined by quantitative immunoblotting (Figure 4A). When OA was supplemented into Aurora A- or Cdc2-depleted interphase extracts, Ser10 phosphorylation and chromatin association of condensin I subunits were indistinguishable from those in the undepleted extract supplemented with OA.
Moreover, Aurora A or Cdc2 depletion did not interfere with the partial condensation of sperm chromatin in the presence of OA (Figure 4Cc, e and f). On the other hand, Ser10 phosphorylation and chromatin association of condensin I were almost abolished in the OA-treated interphase extract that had been depleted of Aurora B (Figure 4B, lane 7). Furthermore, the chromatin structure assembled in this extract was round (Figure 4Cd), similar to that assembled in the interphase extract (Figure 4Cb). From these results, it is strongly suggested that Aurora B is the corresponding kinase that brings about Ser10 phosphorylation, chromatin binding of condensin I and partial condensation of sperm chromatin.

Finally, we examined the supercoiling activity of condensin I in the OA-treated interphase extract because chromosome-like structures assembled in the extract were very fragile and totally differ from chromosomes assembled in the mitotic extract (Figure 5). Condensin I purified from the OA-treated interphase extract has low or undetectable supercoiling activity (Figure 5, upper, lanes 10–13), which was effectively the same as that of the inactive interphase condensin I (Figure 5, upper, lanes 6–9). DNA-binding activity of condensin I was almost constant under all conditions (Figure 5, lower).

**DISCUSSION**

Aurora B is the serine/threonine protein kinase that regulates many processes during cell division, such as chromosome condensation and cohesion, bipolar chromosome attachment, spindle checkpoint and cytokinesis (20,21). Aurora B phosphorylates histone H3 at Ser10, which has long been noted to be correlated with mitosis, both spatially and temporally. Ser10 phosphorylation during mitosis is conserved in a wide range of eukaryotes, and is believed to be closely associated with the progress of mitosis, including chromosome condensation (40); however, recent studies have revealed that the relationship between Aurora B and chromosome condensation, or Ser10 phosphorylation and chromosome condensation is controversial (20,21).

In this study, we extensively analyzed the role of Aurora B during mitosis on chromosome condensation using a Xenopus in vitro system. We showed that Aurora B depletion from Xenopus egg extract did not affect the mitotic chromosome structure in the absence of Ser10 phosphorylation, consistent with previous reports (30,31). In contrast, the amount of condensin I associated with chromosomes decreased to 50% of that in the undepleted extract, by the depletion of Aurora B from mitotic egg extract. Furthermore, a substantial amount of condensin I...
The regulation of the condensin II recruitment to chromosomes is an object of our next interest.

We considered three possibilities for the partial chromosomal targeting of condensin I induced by Aurora B. One is that Ser10 phosphorylation of nucleosomal H3 by Aurora B is implicated during the process (40). Phosphorylated Ser10 of H3 may act as a direct receptor for condensin I binding; for example, it is reported that condensin I is colocalized with phosphorylated H3 during the early stage of chromosome condensation (41). Very recently, it was proposed that H3 phosphorylation is required for the dissociation of HP1 during mitosis (42,43); therefore, it is possible that the local decondensation induced by HP1 dissociation could help condensin I to access chromatin DNA. The second possibility is that chromosomal Aurora B may act as a direct ligand for condensin I binding, or Aurora B could work as condensin I cargo to the chromosomes; however, we have not yet detected the interaction of condensin I and Aurora B. The third possibility is that Aurora B phosphorylates condensin I and phosphorylation may be involved in condensin I binding to chromosomes. Aurora B is able to phosphorylate condensin I in vitro. (data not shown). Alternatively, Aurora B may phosphorylate some proteins that induce the chromosomal targeting of condensin I, or Aurora B may inactivate inhibitors that prevent the chromosomal targeting of condensin I. The next question is why chromosomes were normally condensed in the Aurora B-depleted mitotic extract, despite the amount of condensin I attached to chromosomes being reduced. We consider that discrepancy results from a large stockpile of protein components, which constitute the chromosome structure, such as condensin I in the Xenopus egg extract; therefore, the 50% reduction of chromosomal condensin I may not affect apparent chromosome condensation. In fact, mitotic chromosomes were assembled normally in the mitotic extract that was depleted of 75% of condensin I (data not shown). It is possible that the consequence of Aurora B depletion differs depending on the species, and the developmental stage may come from differences in the concentration of condensin I in the cells.

It is also noted that all of our experiments were performed using a high-speed supernatant, in which no DNA replication occurs, just to focus on the mechanism of the chromosomal association of condensin I. The use of a low-speed supernatant, as described by Cuvier and Hirano, may reflect the more physiological process for chromosome condensation because it allows one round of replication before mitosis (35). It was reported that chromosomal targeting of condensin I and chromosome condensation took place in a low-speed supernatant that was depleted of Aurora B (31) as well as in a high-speed supernatant (35). Precise analysis of the effect of Aurora B depletion on the amount of chromosome bound condensin I in a low-speed supernatant is the next interest of our work.

Finally, we considered two possibilities for OA-dependent partial chromosome condensation in the interphase extract: one is that condensin I bound to sperm chromatin drives chromosome condensation, and the other is that factors other than condensin I are
implicated in condensation. It is reported that the putative factor, RCA, is able to compact chromosome structures without condensin, and is inactivated by PP1 (39), which supports the latter model. But the former model is likely to account for the OA-dependent partial chromosome condensation in this experimental system because chromosome condensation was abolished when condensin I was depleted from OA-treated interphase extract. However, the chromosome-like structures assembled in the OA-treated interphase extract are very fragile and easily collapsed in the presence of high salt. It is noted that the supercoiling activity of condensin I purified from the extract was very weak, indistinguishable from that purified from the interphase extract (Figure 5). Thus, chromosome-like structures may be assembled in the absence of supercoiling activity of condensin I; however, the supercoiling activity of condensin I is required to give structural integrity to the assembled chromosomes. Alternatively, we cannot exclude the possibility that the amount of condensin I associated with chromosomes in the OA-treated interphase extract are too small to make rigid structures.

SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS
This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and from the Solution Oriented Research for Science and Technology (SORST) from the Japan Science and Technology Agency. This work was also supported by the Bioarchitect II Research Project of RIKEN. Funding to pay the Open Access publication charges for this article was provided by grants from the Ministry of Education, Culture, Sports, Sciences and Technology of Japan.

Conflict of interest statement. None declared.

REFERENCES
1. Belmont, A.S. (2002) Mitotic chromosome scaffold structure: new approaches to an old controversy. Proc. Natl. Acad. Sci. USA, 99, 15855–15857.
2. Koshland, D. and Strunnikov, A. (1996) Mitotic chromosome condensation. Annu. Rev. Cell Dev. Biol., 12, 305–333.
3. Wang, J.C. (1996) DNA topoisomerases. Annu. Rev. Biochem., 65, 635–692.
4. Hirano, T., Kobayashi, R. and Hirano, M. (1997) Condensins, chromosome condensation protein complexes containing XCAP-C, XCAP-E and a Xenopus homolog of the Drosophila Barren protein. Cell, 89, 511–521.
5. Hirano, T. and Mitchison, T.J. (1994) A heterodimeric coiled-coil protein required for mitotic chromosome condensation in vitro. Cell, 79, 449–458.
6. Haggerstrom, K.A. and Meyer, B.J. (2003) Condensin and cohesin: more than chromosome compactor and glue. Nat. Rev. Genet., 4, 530–534.
7. Hirano, T. (2000) Chromosome cohesion, condensation, and separation. Annu. Rev. Biochem., 69, 115–144.
8. Hirano, T. (2005) Condensins: organizing and segregating the genome. Curr. Biol., 15, R265–R275.
9. Nasmyth, K. and Haering, C.H. (2005) The structure and function of SMC and kleisin complexes. Annu. Rev. Biochem., 74, 595–648.
10. Kimura, K. and Hirano, T. (1997) ATP-dependent positive supercoiling of DNA by 13S condensin: a biochemical implication for chromosome condensation. Cell, 90, 625–634.
11. Hirano, T., Rybenkov, V.V., Crisoma, N.J., Hirano, T. and Cozzarelli, N.R. (1999) 13S condensin actively reconfigures DNA by introducing global positive writhe: implications for chromosome condensation. Cell, 98, 239–248.
12. Takemoto, A., Kimura, K., Yokoyama, S. and Hanaoka, F. (2004) Cell cycle-dependent phosphorylation, nuclear localization, and activation of human condensin. J. Biol. Chem., 279, 4551–4559.
13. Kimura, K., Hirano, M., Kobayashi, R. and Hirano, T. (1998) Phosphorylation and activation of 13S condensin by Cdc2 in vitro. Science, 282, 487–490.
14. Takemoto, A., Kimura, K., Yanagisawa, J., Yokoyama, S. and Hanaoka, F. (2006) Negative regulation of condensin I by CK2-mediated phosphorylation. EMBO. J., 25, 5339–5348.
15. Ono, T., Losada, A., Hirano, M., Myers, M.P., Neuwald, A.F. and Hirano, T. (2003) Differential contributions of condensin I and condensin II to mitotic chromosome architecture in vertebrate cells. Cell, 115, 109–121.
16. Yeong, F.M., Bomhauer, H., Wendi, K.S., Hirota, T., Mudrak, I., Mechler, K., Loregger, T., Marchler-Bauer, A., Tanaka, K., Peters, J.M. et al. (2003) Identification of a subunit of a novel Kleisin-beta/SMC complex as a potential substrate of protein phosphatase 2A. Curr. Biol., 13, 2058–2064.
17. Gerlich, D., Hirota, T., Koch, B., Peters, J.M. and Ellenberg, J. (2006) Condensin I stabilizes chromosomes mechanically through a dynamic interaction in live cells. Curr. Biol., 16, 333–344.
18. Hirota, T., Gerlich, D., Koch, B., Ellenberg, J. and Peters, J.M. (2004) Distinct functions of condensin I and II in mitotic chromosome assembly. J. Cell Sci., 117, 6435–6445.
19. Sutani, T., Yuasa, T., Tomonaga, T., Dohmae, N., Takio, K. and Yanagida, M. (1999) Fission yeast condensin complex: essential roles of non-SMC subunits for condensation and Cdc2 phosphorylation of Cut3/SMC4. Genes Dev., 13, 2271–2283.
20. Carmena, M. and Earnshaw, W.C. (2003) The cellular geography of aurora kinases. Nat. Rev. Mol. Cell. Biol., 4, 842–854.
21. Meraldi, P., Honda, R. and Nigg, E.A. (2004) Aurora kinases link chromosome segregation and cell division to cancer susceptibility. Curr. Opin. Genet. Dev., 14, 29–36.
22. Gassmann, R., Carvalho, A., Henzing, A.J., Ruchaud, S., Hudson, D.F., Honda, R., Nigg, E.A., Gerloff, D.L. and Earnshaw, W.C. (2004) Borealin: a novel chromosome passenger protein required for stability of the bipolar mitotic spindle. J. Cell Biol., 166, 179–191.
23. Sampath, S.C., Ohi, R., Leismann, O., Salic, A., Pozniakovsky, A. and Funabiki, H. (2004) The chromosomal passenger complex is required for chromatin-induced microtubule stabilization and spindle assembly. Cell, 118, 187–202.
24. Li, X., Sakashita, G., Matsuizaki, H., Sugimoto, K., Kimura, K., Hanaoka, F., Taniguchi, H., Furukawa, K. and Urano, T. (2004) Direct association with inner centromere protein (INCENP) activates the novel chromosomal passenger protein, Aurora-C. J. Biol. Chem., 279, 47201–47211.
25. Morishita, J., Matsuoka, T., Goshima, G., Nakamura, T., Tatebe, H. and Yagunida, M. (2001) Brl1/Cut17 moving from chromosome to spindle upon the loss of cohesion is required for condensation, spindle elongation and repair. Genes Cells, 6, 743–763.
26. Petersen, J. and Hagan, J.M. (2003) S. pombe aurora kinase/survivin is required for chromosome condensation and the spindle checkpoint attachment response. Curr. Biol., 13, 590–597.
27. Giet, R. and Glover, D.M. (2001) Drosophila aurora B kinase is required for histone H3 phosphorylation and condensin recruitment during chromosome condensation and to organize the central spindle during cytokinesis. J. Cell Biol., 152, 669–682.
28. Kaitna, S., Pasierbek, P., Jantsch, M., Lodl, J. and Glotzer, M. (2002) The aurora B kinase AIR-2 regulates kinetochore dynamics during mitosis and is required for separation of homologous chromosomes during meiosis. Curr. Biol., 12, 798–812.
29. Hagstrom, K.A., Holmes, V.F., Cozzarelli, N.R., and Meyer, B.J. (2002) C. elegans condensin promotes mitotic chromosome architecture, centromere organization, and sister chromatid segregation during mitosis and meiosis. *Genes Dev.*, **16**, 729–742.

30. MacCallum, D.E., Losada, A., Kobayashi, R., and Hirano, T. (2002) ISWI remodeling complexes in Xenopus egg extracts: identification as major chromosomal components that are regulated by INCENP-aurora B. *Mol. Biol. Cell*, **13**, 25–39.

31. Losada, A., Hirano, M., and Hirano, T. (2002) Cohesin release is required for sister chromatid resolution, but not for condensin-mediated compaction, at the onset of mitosis. *Genes Dev.*, **16**, 3004–3016.

32. Sawin, K.E., Mitchison, T.J., and Wordeman, L.G. (1992) Evidence for kinesin-related proteins in the mitotic apparatus using peptide antibodies. *J. Cell Sci.*, **101**(Pt 2), 303–313.

33. Losada, A., Hirano, M., and Hirano, T. (1998) Identification of Xenopus SMC protein complexes required for sister chromatid cohesion. *Genes Dev.*, **12**, 1986–1997.

34. Losada, A., Yokochi, T., Kobayashi, R., and Hirano, T. (2000) Identification and characterization of SA/Sc3p subunits in the Xenopus and human cohesin complexes. *J. Cell Biol.*, **150**, 405–416.

35. Cuvier, O. and Hirano, T. (2003) A role of topoisomerase II in linking DNA replication to chromosome condensation. *J. Cell Biol.*, **160**, 645–655.

36. Hu, J.Y., Sun, Z.W., Li, X., Reuben, M., Tatchell, K., Bishop, D.K., Gruschow, J.M., Bramc, C.J., Caldwell, J.A., et al. (2000) Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glic/PP1 phosphatase in budding yeast and nematodes. *Cell*, **102**, 279–291.

37. Ono, T., Fang, Y., Spector, D.L., and Hirano, T. (2004) Spatial and temporal regulation of Condensins I and II in mitotic chromosome assembly in human cells. *Mol. Biol. Cell*, **15**, 3296–3308.

38. Lavoie, B.D., Hogan, E., and Koshland, D. (2004) In vivo requirements for rDNA chromosome condensation reveal two cell-cycle-regulated pathways for mitotic chromosome folding. *Genes Dev.*, **18**, 76–87.

39. Vagnarelli, P., Hudson, D.F., Ribeiro, S.A., Trinkle-Mulcahy, L., Spence, J.M., Lai, F., Farr, C.J., Lamond, A.I., and Earnshaw, W.C. (2006) Condensin and Repo-Man-PP1 co-operate in the regulation of chromosome architecture during mitosis. *Nat. Cell Biol.*, **8**, 1133–1142.

40. Hans, F. and Dimitrov, S. (2001) Histone H3 phosphorylation and cell division. *Oncogene*, **20**, 3021–3027.

41. Schmiesing, J.A., Gregson, H.C., Zhou, S., and Yokomori, K. (2000) A human condensin complex containing hCAP-C-hCAP-E and CNAP1, a homolog of Xenopus XCAP-D2, colocalizes with phosphorylated histone H3 during the early stage of mitotic chromosome condensation. *Mol. Cell Biol.*, **20**, 6996–7006.

42. Fischle, W., Tseng, B.S., Dormann, H.L., Ueberheide, B.M., Garcia, B.A., Shabanowitz, J., Hunt, D.F., Funabiki, H., and Allis, C.D. (2005) Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. *Nature*, **438**, 1116–1122.

43. Hirota, T., Lipp, J.J., Toh, B.H., and Peters, J.M. (2005) Histone H3 serine 10 phosphorylation by Aurora B causes HP1 dissociation from heterochromatin. *Nature*, **438**, 1176–1180.