Expression and Functional Dynamics of the XCAP-D2 Condensin Subunit in *Xenopus laevis* Oocytes*

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The 13 S condensin complex plays a crucial role in the condensation and segregation of the two sets of chromosomes during mitosis in vivo as well as in cell-free extracts. This complex, conserved from yeast to human, contains a heterodimer of structural maintenance of chromosome (SMC) family proteins and three additional non-SMC subunits. We have investigated the expression of the non-SMC condensin component XCAP-D2 in *Xenopus laevis* oocytes. When studied during meiotic maturation, XCAP-D2 starts to accumulate at the time of germinal vesicle breakdown and reaches its maximal amount in metaphase II oocytes. This accumulation is specifically blocked by injection of antisense oligonucleotides. XCAP-D2 antisense-injected oocytes progress normally through meiois until metaphase II. At this stage, however, chromosomes exhibit architecture defaults, and resolution of sister chromatids is impaired. Surprisingly, in mitotic extracts made from XCAP-D2 knocked-down oocytes, sperm chromatin normally condenses into compacted chromosomes, whereas the amounts of both free and chromosome-bound XCAP-D2 are markedly reduced. This apparent discrepancy is discussed in light of current knowledge on chromosome dynamics.

During mitosis, replicated genomes must be equally distributed between daughter cells. This implies proper segregation of sister chromatids, a process that in turn requires the chromatins to be condensed into compact mitotic chromosomes. The condensation and segregation of chromosomes require several activities, and among them is that of 13 S condensin, a pentameric complex that supports DNA-dependent ATPase activity and is able to introduce positive supercoils into DNA in the presence of ATP (1, 2). The condensin subunits are conserved in a wide variety of species from yeast to mammals. In *Xenopus laevis*, they are given the name of XCAP-C, XCAP-E, XCAP-D2, XCAP-G, and XCAP-H. XCAP-C and XCAP-E belong to the structural maintenance of chromosomes (SMC) family whose homologues are Smc4p and Smc2p in budding yeast (3).

Numerous biochemical studies of the condensin components have been made in *Xenopus* egg extracts. In addition to the complete 13 S condensin complex, subcomplexes have been described that contain some but not all subunits: an 8 S subcomplex composed of the two SMC subunits and a much less abundant 11 S subcomplex composed of the three non-SMC subunits (4). The non-SMC subunits were shown to be specifically phosphorylated in mitotic extracts, and this phosphorylation is necessary to stimulate supercoiling activity (5). The functional contributions of the different condensin subcomplexes have been investigated by adding each of them back to immunodepleted extracts. These studies have led to the conclusion that condensation activity requires the entire 13 S condensin to be formed by joining both 8 and 11 S subcomplexes (2, 4).

The regulatory 11 S subcomplex is required for targeting condensin to chromosomes in mitotic extracts. This subcomplex is also involved in modulating condensin activity. Whereas the SMC subunits share ATP-binding motifs, 8 S condensin has only poor ATPase activity. Its joining to 11 S condensin leads to the stimulation of both the DNA-dependent ATPase and supercoiling activities (4). For these reasons, 8 and 11 S condensins are considered as core and regulatory subcomplexes, respectively.

Genetic studies in yeast have unambiguously established that all condensin subunits are required for a correct segregation of sister chromatids (6–8). In budding yeast it has been shown that loading of condensin onto chromatin requires the presence of each non-SMC subunit (8). However, the individual roles of these subunits have not been fully documented. For instance, the Barren protein (the *Drosophila* homologue of XCAP-H) was shown to interact with topoisomerase II (topo II) (9), but its possible role in topological separation of sister chromatids was not directly confirmed (10). Recent studies carried out with mammalian cells suggested that XCAP-H in targeting the condensin complex onto chromatin through its interaction with the 95-kDa A kinase-anchoring protein (AKAP95) (11).

The role of XCAP-D2 is even more elusive. Early studies used immunoblotting experiments to address the role of XCAP-D2 in *Xenopus* mitotic extracts. However, the observed effects of anti-XCAP-D2 antibodies on chromosome condensation probably reflected an inhibition of the whole condensin function (12). In human cells, a green fluorescent protein-tagged form of

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CNAP1 (the homologue of XCAP-D2) was reported to be addressed to the nucleus and loaded onto mitotic chromatin independently of other condensin components (13). Domains involved in chromatin binding were shown to interact with H1 and H3 histones (13). However, the eventual role of XCAP-D2 in localizing and/or regulating the whole condensin complex has not yet been elucidated. Here we have studied the expression of XCAP-D2 during oocyte maturation in X. laevis as compared with the SMC subunits of the condensin complex. We showed that XCAP-D2 strongly accumulated during oocyte maturation, whereas the levels of XCAP-E, XCAP-C, and XCAP-H remain approximately constant. We have taken advantage of this expression profile to design antisense oligonucleotides that are able to block XCAP-D2 accumulation when injected into oocytes before maturation. By using this approach, we show that meiotic maturation proceeds normally in XCAP-D2 knock-down oocytes. However, the shape of meiotic chromosomes is altered, and sister chromatids fail to separate in these oocytes. In contrast, chromosome assembly is not markedly affected in mitotic extracts made from XCAP-D2 knock-down oocytes.

**EXPERIMENTAL PROCEDURES**

**Antibodies—**Antisera against XCAP-D2 and XCAP-E were obtained from rabbits immunized by injecting polypeptides corresponding to the C-terminal regions of these proteins (amino acids 1347–1386 and 1190–1203, respectively). Antibodies against XCAP-C (14), monoclonal antibodies against p210 (15), and anti-mouse beta II antibodies (16) were provided by Drs T. Hirano, C. Prigent, and S. Dimitrov, respectively. Anti-XCAP-H antibodies against the full-length recombaint protein expressed in *Escherichia coli* were provided by Drs K. Mae-shima and U. K. Laemmli. Anti-beta-tubulin monoclonal antibodies (Sigma) were used according to the manufacturer’s instructions.

**Protein Extraction from Oocytes—**Batches of 10 oocytes were homogenized in 100 μl of STE buffer (100 mM NaCl, 10 mM Tris-HCl, 1 mM potassium EDTA, pH 7.8) containing a mixture of protease inhibitors (10 μg/ml leupeptin, 10 μg/ml pepstatin, and 10 μg/ml chymostatin). Samples were centrifuged at 10,000 × g for 10 min at 4 °C. Supernatants were extracted with 1/5 of 1.1,3-trichlorofluoroethane and centrifuged at 10,000 × g for 10 min at 4 °C.

**Immunoprecipitation—**Aliquots of oocyte extracts (50 μl) were diluted 10-fold in XB-E2 (100 mM KCl, 50 mM sucrose, 10 mM Hepes, 5 mM EGTA, pH 7.8) and centrifuged at 13,000 × g for 4 °C for 15 min, the supernatants were used for immunoprecipitations. Crude sera (1/100 μl of diluted extract) were added to the clarified extracts and incubated at 4 °C for 3 h. Immunoprecipitates were then recovered by adding protein A-Sepharose beads (1:25, v/v) (Amersham Biosciences) and incubating for 1 h at room temperature with constant agitation. After centrifugation, beads were washed three times with 1 ml of XB-E2 containing 1% Nonidet P-40. Beads were resuspended in 50 μl of Laemmli sample buffer and heated to 90 °C for 10 min. 5 μl of each sample were analyzed by SDS-PAGE and Western blot.

**Western Blot Analysis—**Electrophoresis was performed on SDS-polyacrylamide gels and electrotransfer onto nitrocellulose membranes were performed following standard procedures. Membranes were blocked in TBST (25 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20 (Sigma) containing 5% skimmed milk for 2 h at 4 °C). Membranes were incubated at 4 °C for 1 h with crude sera diluted 1,000-fold in TBST containing 2.5% skimmed milk. Immunocomplexes were revealed with alkaline phosphatase-coupled anti-rabbit antibodies (Jackson ImmunoResearch Laboratories, Inc.) using a chemifluorescence assay (Amersham Biosciences) according to the manufacturer’s instructions. Signals were quantified using ImageQuant (Amersham Biosciences).

**Preparation of Stage VI Oocytes and in Vitro Maturation—**Ovarian follicles were first incubated in OR2 (82.5 mM NaCl, 2.5 mM KCl, 5 mM Hepes, 1 mM Na3HPO4, 1 mM MgCl2, pH 7.8) containing 1 mM CaCl2 and 400 μg/ml disase II (Roche Applied Science) for 3 h at 20 °C. After rinsing several times in OR2, they were incubated at 20 °C in OR2 containing 3.3% formaldehyde and 10 μg/ml DAPI. The matur-ation process was monitored by visual observation under a microscope (Axioskop 2, Carl Zeiss). The matura-tion was considered to be complete when two polar bodies had been ejected (Fig. 1B).

**RESULTS**

**XCAP-D2 Accumulates during Oocyte Maturation—**We investigated the expression of XCAP-D2, XCAP-C, XCAP-E, and XCAP-H during oocyte maturation. Full grown oocytes were matured by incubation in progesterone at 22 °C. The maturation process was followed by monitoring the appearance of a characteristic white spot at the animal pole. This morpholog-ical change is known to mark the germinal vesicle breakdown, an early event in meiotic maturation. Fig. 1B shows that 100% of the oocytes underwent germinal vesicle breakdown between 2 and 8 h of incubation in progesterone. Protein samples were taken at 2-h intervals and analyzed by Western blotting with polyclonal antibodies specific for these proteins (Fig. 1A). Levels of XCAP-C and XCAP-E remained constant all along the maturation process. In contrast, XCAP-D2 was barely detectable until 4 h of incubation, and then it strongly accumulated. Quantification of XCAP-D2 signals showed that the accumula-tion of XCAP-D2 continued between 8 and 12 h, a period of time where 100% of the oocytes had undergone germinal vesicle breakdown (Fig. 1B, GVBD). Several independent experiments showed that the accumulation of XCAP-D2 stopped after about 15 h when the level of this protein became comparable to that observed in unfertilized eggs. Cytological observation of oocytes stained with DAPI showed that, after 12 h of incubation in progesterone, all of them had ejected the first polar body (data not shown). The XCAP-D2 level remained constant when oocytes were incubated for 12 h in the absence of progesterone (Fig. 1A, compare lanes 1 and 8). The expression of XCAP-D2 was further compared with that of XCAP-H, another non-SMC subunit of condensin (Fig. 1C). The amount of XCAP-H was found to increase much less strongly than that of XCAP-D2.
These data indicate that the expression levels of condensin subunits are differentially regulated during oocyte maturation.

Newly Synthesized XCAP-D2 Is Recruited into the Condensin Complex—Xenopus eggs were shown to contain both the 8 and 13 S condensin complexes (1). The 8 S core subcomplex is a heterodimer composed of XCAP-C and XCAP-E, whereas the 13 S holocomplex contains three additional subunits, XCAP-D2, XCAP-G, and XCAP-H. Accordingly the presence of XCAP-D2 in immunoprecipitates obtained with anti-XCAP-E antibodies can be taken as evidence of the 13 S complex. The assembly of 13 S condensin was therefore investigated during oocyte maturation by immunoprecipitation. Samples taken at different times after progesterone addition were immunoprecipitated with anti-XCAP-E antibodies and analyzed by Western blotting with anti-XCAP-C, anti-XCAP-E, and anti-XCAP-D2 antibodies. As shown in Fig. 2A, basal levels of XCAP-D2 present in non-mature oocytes were already associated with a complex that contained XCAP-C and XCAP-E (lanes 1 and 8). During the time course of maturation (lanes 2–7), the newly synthesized XCAP-D2 was recruited into this complex (compare Figs. 1A and 2A). That the recruitment of XCAP-D2 proceeded as long as it accumulated suggested that, even in fully matured oocytes, XCAP-D2 was not in excess with respect to XCAP-C

![Fig. 1. Expression of condensin subunits during meiotic maturation. A, groups of 10 oocytes were incubated with (+) or without (−) progesterone and collected at the indicated times. Proteins were extracted and analyzed by Western blot using anti-XCAP-C, anti-XCAP-E, and anti-XCAP-D2 antibodies. B, levels of XCAP-D2 (black dots) were quantified at the indicated times after progesterone treatment and expressed as percentages of the level present in mature oocytes. The percentage of germinal vesicle breakdown (GVBD) was monitored from a sibling batch of oocytes and plotted on the same graph (open circles). C, groups of 10 oocytes were incubated with progesterone (Pg) and collected at the indicated times. Proteins were extracted and analyzed by Western blot using anti-XCAP-D2 and anti-XCAP-H antibodies](http://www.jbc.org/)

![Fig. 2. Recruitment of XCAP-D2 into the condensin complex during oocyte maturation. A, oocytes were incubated with (+) or without (−) progesterone for the indicated times. Proteins were extracted and immunoprecipitated with anti-XCAP-E antibodies. Immunoprecipitates were analyzed by Western blot using anti-XCAP-C, anti-XCAP-E, and anti-XCAP-D2 antibodies. B, proteins were extracted from stage VI oocytes (upper panel) or oocytes that had been matured in vitro by progesterone treatment (lower panel). These extracts were fractionated by ultracentrifugation through a 5–20% sucrose gradient. Collected fractions were analyzed by Western blot using anti-XCAP-E, anti-XCAP-D2, and anti-XCAP-H antibodies. Bovine serum albumin (4.6 S, fraction 6) and catalase (11.3 S, fraction 13) were centrifuged in a separate gradient and used as sedimentation markers. C, amounts of XCAP-D2 (open squares) and XCAP-E (black squares) in stage VI (upper panel) or matured (lower panel) oocytes were quantified in each fraction and plotted as a percentage of the total amount present in matured oocytes. Pg, progesterone; D2, XCAP-D2; E, XCAP-E; H, XCAP-H.](http://www.jbc.org/)
and XCAP-E. To confirm this and to investigate the stoichiometry of these complexes, extracts made from mature and immature oocytes were analyzed by sucrose gradient centrifugation (Fig. 2, B and C). In immature oocytes (upper panels), XCAP-D2 was recovered in fractions 14 and 15 corresponding to sedimentation coefficients close to 13 S. Since these fractions also contained both XCAP-E and XCAP-H, they probably correspond to 13 S condensin. A major part of XCAP-E and XCAP-H was recovered in fractions 10–12 whose sedimentation coefficient was lower than 11.3 S. Although these proteins partially cofractionated, maximal amounts of XCAP-E were found in fractions 10–11, whereas XCAP-H peaked in fraction 12. Accordingly fractions 10–11 would contain 8 S condensin, whereas fraction 12 could correspond to another complex containing XCAP-H but devoid of XCAP-D2. In mature oocytes (Fig. 2, B and C, lower panels), the main part of XCAP-E and about 75% of XCAP-H was shifted to fractions higher than 11.3 S (fractions 14–17). According to their sedimentation coefficients, these fractions, which also contain newly synthesized XCAP-D2, probably correspond to the 13 S condensin complex. Taken together, these data indicate that the accumulated XCAP-D2 was entirely recruited into the 13 S condensin complex, confirming that this protein is not in excess with respect to the other condensin subunits in mature oocytes.

Antisense Oligonucleotides Block XCAP-D2 Synthesis and 13 S Condensin Accumulation in Mature Oocytes—We reasoned that antisense oligonucleotides should inhibit XCAP-D2 expression by translationally inactivating its mRNA. Four adjacent sense/antisense oligonucleotide couples were derived from the 5′-untranslated region of XCAP-D2 mRNA (Fig. 3A). The 5′-untranslated region of XCAP-D2 mRNA is presented. Positions of the four sense/antisense oligonucleotide couples are underlined. The initiation codon is boxed. Oligonucleotide couples are named S1/AS1 to S4/AS4 from the 5′-end to the initiation codon. B, oocytes were injected with a mixture of the four sense (S) or antisense (AS) oligonucleotides and incubated with (+) or without (−) progesterone. Proteins were extracted and analyzed by Western blot with anti-XCAP-D2 and anti-pEg2 antibodies. C, oocytes were injected with sense (S1 to S4) or antisense (AS1 to AS4) oligonucleotides and incubated with progesterone. Proteins were extracted and analyzed by Western blot with anti-XCAP-D2 and anti-pEg2 antibodies. D, oocytes were injected with S3 or AS3 oligonucleotides and incubated with (+) or without (−) progesterone. Proteins were extracted and analyzed by Western blot with anti-XCAP-D2 and anti-pEg2 antibodies. E, the amounts of XCAP-D2 and pEg2 were quantified and plotted as a percentage of the amounts of these proteins present in non-injected mature oocytes. F, oocytes were injected with S3 or AS3 oligonucleotides. Proteins were extracted, separated by ultracentrifugation through a 5–20% sucrose gradient, and analyzed by Western blot with anti-XCAP-E and anti-XCAP-H antibodies. The position of catalase (11.3 S) is indicated.
expression of XCAP-D2 and pEg2 were analyzed after progesterone treatment (Fig. 3C). Antisense oligonucleotides AS1, AS3, and AS4 were found to efficiently inhibit XCAP-D2 synthesis, whereas the corresponding sense oligonucleotides had no effect. Oligonucleotide AS2 was found to be inefficient, whereas surprisingly the corresponding sense oligonucleotide (S2) partially affected XCAP-D2 accumulation. The synthesis of pEg2 was also diminished in oocytes that had been injected with S2 indicating that the effect of this oligonucleotide on XCAP-D2 synthesis was not specific. Among the antisense oligonucleotides that did affect the expression of XCAP-D2, AS3 had the least effect on the expression of pEg2. The specificity of the S3/AS3 oligonucleotide couple was analyzed in more detail in Fig. 3D and E. The expression of XCAP-D2 was not affected by S3 oligonucleotide as compared with non-injected oocytes (compare lanes 2 and 3). When oocytes were injected with AS3 oligonucleotides, XCAP-D2 levels remained close to those observed in immature oocytes (compare lanes 1 and 4). Considering the total amount of XCAP-D2, this corresponded to a depletion of more than 95%. In contrast, neither S3 nor AS3 diminished levels of pEg2 that accumulate in mature oocytes. From these observations, the S3/AS3 couple was retained for further experiments. Since newly synthesized XCAP-D2 was found to be entirely recruited into the condensin complex, we compared the sedimentation behavior of condensin subunits in oocytes injected with either S3 or AS3 oligonucleotides and matured. For this purpose, protein extracts of both series were analyzed by sucrose density gradients (Fig. 3F). In sense-injected oocytes (upper panel), XCAP-E was distributed between the 8 S (fractions 10–11) and 13 S (fractions 15–17) complexes. This is consistent with previous reports showing that 8 and 13 S condensin complexes coexist in unfertilized eggs (1). As in Fig. 2, XCAP-H was recovered in fractions corresponding to both the 13 S condensin (fractions 15–17) and complexes of lower densities. In antisense-injected oocytes (lower panel), the majority of both XCAP-E and XCAP-H were recovered in fractions whose sedimentation coefficients were lower than 13 S. This indicates that accumulation of 13 S condensin is prevented in XCAP-D2 knock-down oocytes.

**Effect of XCAP-D2 Knock-down on Meiotic Chromosomes**—To investigate the role of XCAP-D2 in the dynamics of meiotic chromosomes, oocytes were injected with sense or antisense oligonucleotides, matured, fixed in DAPI-containing methanol, and directly observed through the depigmented maturation spot. In these conditions both the first polar body and metaphase chromosomes were observable (Fig. 4A). Although not always present in the same optical field as metaphase chromosomes, the first polar body was observed in all the oocytes of both series, indicating that they had correctly passed the first meiotic division. Moreover chromosomes were correctly aligned on the metaphase plate in sense- as well as in antisense-injected oocytes. Although their overall compactness was not markedly affected, chromosomes of antisense-injected oocytes appeared thicker and rounder than those of sense-injected oocytes. The occurrence of this altered, ovoid shape was scored in both series (Fig. 4B), and it was confirmed that there were significantly more ovoid shaped chromosomes in antisense- than in sense-injected oocytes. A low number of ovoid chromosomes is also detected in the control oocytes. Since similar pictures were observed in non-injected oocytes (data not shown), this most probably reflects naturally occurring variations in either the shape or orientation of meiotic chromosomes.

In mitotic cells, the cohesin complex dissociates from the chromosome arms by a proteolysis-independent pathway before the onset of anaphase (22, 23). This potentially allows the separation of sister chromatids to be observed by simply extending the incubation in progesterone. In preliminary experiments, we showed that when full grown oocytes were observed 20 h after progesterone addition sister chromatids spontaneously separated from each other at several locations along the chromosome arms (data not shown). This spontaneous separation of sister chromatids was subsequently analyzed in oocytes that had been injected with sense or antisense oligonucleotides...
and then incubated with progesterone for 20 h (Fig. 4C). In sense-injected oocytes, a local separation of sister chromatids was visible on several chromosomes as either forks or bubbles (panel a, see arrowheads). On the contrary, such events were not observed in antisense-injected oocytes (panel b), suggesting that the separation of sister chromatids is impaired in the absence of newly synthesized XCAP-D2.

Assembly of Chromosomes in Mitotic Extracts Made from XCAP-D2 Knocked-down Oocytes—When incubated in mitotic extracts prepared from unfertilized eggs, sperm chromatin undergoes local condensation and finally resolves into highly condensed individual chromosomes (18, 24). To investigate the potential role of newly synthesized XCAP-D2 in this process, mitotic extracts were prepared from mature oocytes injected with sense or antisense oligonucleotides. As expected from the data in Fig. 3D, the amount of XCAP-D2 was reduced by more than 95% in extracts made from knocked-down oocytes as compared with control extracts (Fig. 5A). Aliquots of the same extracts were incubated for 2 h with sperm chromatin and observed by fluorescence microscopy (Fig. 5B). Surprisingly no obvious difference was detected between extracts made from control or antisense-injected oocytes (Fig. 5B, compare panels a and b with panels c and d). In both extracts, the chromatin efficiently condensed, forming fibrous masses of compacting DNA. Chromatin fibers were found to be slightly thicker in extracts made from antisense-injected oocytes (compare panels a and c). However, the assembly of fully compact, individualized chromosomes occurred with the same efficiency in both extracts (compare panels b and d). Interestingly, in some experiments, the individualization of chromosomes was even more efficient in extracts made from antisense-injected oocytes. These observations indicate that chromosome assembly is not impaired in extracts made from XCAP-D2 knocked-down oocytes.

Chromosome-associated Proteins in Extracts Made from XCAP-D2 Knocked-down Oocytes—A possible interpretation of the above observations is that XCAP-D2 is not required for chromosome assembly in mitotic extracts. However, due to residual XCAP-D2, it cannot be ruled out that even in extracts made from antisense-injected oocytes the amount of XCAP-D2 loaded onto chromosomes remains unchanged. To test this hypothesis, mitotic extracts were made from injected oocytes and incubated with sperm chromatin. After 2 h of incubation, free and chromatin-bound proteins were separated by centrifugation through a 30% sucrose cushion (Fig. 6). Preliminary experiments showed that, in the absence of oocyte extracts, purified sperm chromatin contained no detectable levels of either XCAP-E or XCAP-D2 (data not shown). No detectable level of XCAP-E was found in pellets obtained in the absence of sperm chromatin (Fig. 6, lanes 2 and 4). In the presence of sperm chromatin, a small amount of XCAP-E was associated with the chromatin pellet. Taking into account the differential dilution of the free and bound fractions (see “Experimental Procedures”), this represents about 6% of total XCAP-E (Fig. 6, lane 6). When similarly quantified, the amount of XCAP-D2 that was associated with chromatin corresponded to less than 3% of the total. Since XCAP-D2 is not found to be in excess with respect to XCAP-E (see Fig. 2), this suggests that a larger amount of XCAP-E than XCAP-D2 is loaded onto chromatin. In extracts made from antisense-injected oocytes, the amount of chromosome-bound XCAP-D2 was strongly diminished, whereas the amounts of both XCAP-E and topoisomerase II recovered in the chromosomal fraction were essentially not affected (Fig. 6, compare lanes 6 and 8). Binding of XCAP-H to chromosomes was also investigated. In this experiment, this protein was resolved as a doublet. According to previous studies (5), this could be due to the phosphorylation of XCAP-H in mitotic extracts. Only the form of lower electrophoretic mobility was recovered in the chromosomal fraction (lane 6). In contrast to what was observed for XCAP-E, the amount of chromosome-bound XCAP-H was markedly reduced in knocked-down extracts (compare lanes 6 and 8). Therefore, decreasing the amount of XCAP-D2 has differential effects on the loading of other condensin subunits onto chromosomes.

**DISCUSSION**

We have studied the expression of XCAP-D2, one of the three non-SMC condensin subunits, during meiotic maturation in *X. laevis*. We show that the SMC subunits XCAP-C and XCAP-E are already present in full grown oocytes and that their levels remain constant during oocyte maturation. In contrast, XCAP-D2 is barely detectable in immature oocytes but strongly accumulates upon maturation. XCAP-H, another non-SMC subunit, is abundant in immature oocytes, and its expression increases by less than 2-fold upon maturation.

The accumulation of XCAP-D2 during oocyte maturation is likely due to translational recruitment of its maternal mRNA. Translational control of maternally inherited mRNAs was shown to play an important role in the oogenesis and early development of numerous metazoans. In *Xenopus*, maternal mRNAs whose 3'-untranslated region contains cytoplasmic
polyadenylation elements (CPEs; consensus sequence: UUUUUAA_{1-6}U) are concomitantly polyadenylated and recruited into polyosomes during oocyte maturation (for review, see Ref. 25). CPEs also repress the translation of maternal mRNAs in immature oocytes (26). The presence of four putative CPEs (UUUUUUAAU, UUUUUAAU, UUUUUAAU, and UUUUUAAU at positions 4281, 4304, 4333, and 4352, respectively) in the 3′-untranslated region of the XCAP-D2 maternal mRNA (GenBank\textsuperscript{TM} accession number AF067969;5) probably accounts for both the repression of its expression in immature oocytes and its accumulation upon oocyte maturation. It is to be noted that XCAP-E mRNA (GenBank\textsuperscript{TM} accession number U13674) contains only one putative CPE (UUUUUAU) located immediately upstream of the putative nuclear polyadenylation signal, whereas no consensus CPE is found in the 3′-untranslated regions of XCAP-C, XCAP-G, and XCAP-H (GenBank\textsuperscript{TM} accession numbers U13673, AF111423, and U90125, respectively). We did not obtain anti-XCAP-G antibodies, which would have permitted us to study the expression profile of this protein. However, given the absence of putative CPE in the corresponding mRNA, one can extrapolate that XCAP-D2 is probably the only condensin subunit whose expression increases by more than 10-fold upon maturation.

In stage VI oocytes, XCAP-E and XCAP-H are primarily present in complexes whose sedimentation coefficients are comprised between 8 and 10 S. Since XCAP-E is present in fractions whose density is close to 8 S, this subunit is probably associated with XCAP-C to form an 8 S condensin complex. Basal levels of XCAP-D2 are present in fractions corresponding to sedimentation coefficients close to 13 S. As these fractions also contain XCAP-E and XCAP-H, they probably contain the small amount of already formed 13 S condensin. What the function of condensin subunits could be in stage VI oocytes remains unknown. We have observed that about 25% of XCAP-C and XCAP-E is localized inside the germinal vesicle, the remaining being cytoplasmic.\textsuperscript{2} One might hypothesize that these proteins participate in the structural maintenance of the lambrush chromosomes. At the onset of oocyte maturation, they could also be required to initiate the condensation of meiotic chromosomes. Alternatively SMC proteins could play a role in the transcriptional regulation of the maternal genome in oocytes. Since XCAP-D2 is not in excess with respect to 13 S complex, each non-SMC condensin subunit was shown to be required to initiate the condensation of the X chromosome in hermaphrodite Caenorhabditis elegans (27), the transcriptional repression of the Polycomb complex in Drosophila melanogaster (28), and in silencing of mating type loci in budding yeast (29).

During oocyte maturation, the amount of XCAP-D2 increases more than 10-fold and is stoichiometrically recruited into SMC-containing complex whose sedimentation coefficient shifts to 13 S. At the same time, XCAP-H is also recruited into this complex. As a consequence the amount of 13 S condensin increases as XCAP-D2 accumulates. At the end of maturation, XCAP-D2 is not in excess with respect to 13 S complex, whereas about 25% of XCAP-H remains in complexes of lower density. As reported previously (1), XCAP-E is present in both 8 and 13 S condensins in mature oocytes. Taken together, these data suggest that XCAP-D2 is a limiting factor for the formation of 13 S condensin during oocyte maturation.

We have taken advantage of the dynamic accumulation of XCAP-D2 during oocyte maturation to prevent its expression by injecting antisense oligonucleotides. These have dramatic effects on the expression of XCAP-D2, the level of which remains close to that observed in non-mature oocytes. As a consequence, 13 S condensin fails to accumulate, and a major part of both XCAP-E and XCAP-H remains in complexes of lower sedimentation coefficients, close to those observed in immature oocytes.

The effects of XCAP-D2 knock-down on chromosome dynamics were investigated in living oocytes. This had no visible consequences on the ongoing oocyte maturation, namely the ejection of the first polar body (reflecting that meiosis I proceeded normally) and the organization of the metaphase plate in meiosis II. This is in agreement with previous studies showing that, in C. elegans, condensin is dispensable in meiosis I (30, 31). In meiosis II, however, chromosome architecture is clearly affected in XCAP-D2 knocked-down oocytes. Whereas their overall condensation level is only moderately affected, they are much more rounded than in control oocytes.

Another clear effect of XCAP-D2 knock-down concerns the proteolysis-independent resolution of sister chromatids that can be observed by extending the maturation time. In contrast to what was observed in control oocytes, sister chromatids were not distinguishable in chromosomes from antisense-injected oocytes.

Both the altered shape of chromosomes and defaults in chromatid resolution observed in XCAP-D2 knocked-down oocytes are consistent with recent reports showing that, in metazoans, condensin is required in loop compaction and sister chromatid resolution rather than in an overall condensation of chromatin. When smc4 (the homologue of XCAP-C) is depleted by RNA interference in C. elegans, chromosomes retain a high degree of condensation during metaphase but have altered morphology, and sister chromatids fail to segregate during meiosis II anaphase as well as in mitotic blastomeres (30, 31). This is very similar to what is observed in Drosophila embryos of glon mutants in which the smc4 homologue is mutated (32). Such altered shapes are likely to reflect defaults in the condensation of chromosome lateral loops (33, 34).

In a previous study (12), we reported that two antibodies directed against two adjacent parts of XCAP-D2 had distinct effects on chromosome assembly in mitotic extracts. One of them completely impaired the condensation process, giving pictures of uncondensed chromatin that were very similar to those observed in condensin-depleted extracts (1). The effects of this antibody were therefore proposed to reflect an inactivation of the whole condensin complex rather than a strict requirement of XCAP-D2 in this process. The other antibody had much more subtle effects: in the presence of saturating amounts of this antibody, the chromatin condensed at least partially, giving chromosomes that, although being thicker than in control extracts, were clearly distinguishable. In the present study, we have used the antisense approach to directly address the role of XCAP-D2 in mitotic extracts. Surprisingly chromosome assembly from sperm chromatin was not affected in extracts where XCAP-D2 expression had been reduced by up to 95%. This apparent lack of effect was intriguing since immunodepletion experiments had previously demonstrated the role of 13 S condensin in chromosome assembly (1). In Schizosaccharomyces pombe, each non-SMC condensin subunit was shown to be essential not only for sister chromatid separation but also for chromosome condensation (7). Nonetheless, contrary to what happens in mutant yeasts where functional protein is totally absent, mitotic extracts were depleted by only 95% due to basal levels of XCAP-D2 present in immature oocytes. It is therefore conceivable that residual XCAP-D2 would be sufficient with respect to the number of chromosomes to be assembled in condensation assays. However, biochemical analysis of \textit{in vitro} assembled chromosomes revealed that the amount of chromosome-associated XCAP-D2 was markedly diminished in knocked-down extracts.

\textsuperscript{2} F. Cubizolles, unpublished data.
Targeting of XCAP-E (an 8 S condensin subunit) and XCAP-H (an 11 S condensin subunit) onto chromosomes was studied in extracts partially depleted of XCAP-D2. Recruitment of XCAP-E was essentially not affected. A possible explanation is that XCAP-D2 is dispensable for loading the 8 S condensin core complex onto chromosomes. Alternatively the requirement of XCAP-D2 might not be stoichiometric with respect to the amount of condensin recruited onto chromosomes. Accordingly loading only a few intact 13 S condensin molecules onto chromosomes might assist, by a nucleation-like process, the recruitment of additional SMC-containing complexes, eventually devoid of XCAP-D2. As a result, chromatin would contain sufficient amounts of SMC proteins to ensure assembly of mitotic like chromosomes. Contrary to what is observed for XCAP-E, chromosome targeting of XCAP-H, an 11 S condensin subunit, was affected by a partial depletion of XCAP-D2. This observation makes sense if one considers that hCAP-D2 and XCAP-H were found to directly interact with each other in a two-hybrid assay (11).3

Taken together, these observations suggest that, in Xenopus cell-free extracts, the contribution of XCAP-D2 and XCAP-H to condensin loading is not stoichiometric. In contrast, observations made in living cells suggest a direct dependence of condensin recruitment upon several non-SMC subunits including XCAP-D2 and XCAP-H. For instance, XCAP-D2 homologue was shown to be required for chromatin localization of condensin in Saccharomyces cerevisiae (8). Recent studies show that in human cells this protein is able to address itself onto mitotic chromatin through its association with histones (13), an observation that is compatible with a role for XCAP-D2 as a loading factor for condensin. Genetic studies have shown that other condensin subunits are also involved in nuclear and/or chromatid localization (8). Moreover the human homologue of XCAP-H, another condensin subunit related to the Drosophila protein Barren, was shown to be required for the chromatin targeting of condensin in human cells through its association with AKAP95 (11).

According to the above observation together with previous reports, mechanisms involved in chromosomal condensation are likely to be somehow different in mitotic extracts and in living cells. Some of these differences could come from the male origin of the chromatin that is used in these assays. Recent studies have shown that in mouse zygotes chromosome condensation, which does not correspond to any reported in mouse eggs, some of these differences could come from the male origin of the chromatin that is used in these assays. Recent studies have shown that in mouse zygotes chromosome condensation, which does not correspond to any

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Expression and Functional Dynamics of the XCAP-D2 Condensin Subunit in
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