Brief Report

A Kinase–anchoring Protein (AKAP)95 Recruits Human Chromosome-associated Protein (hCAP)-D2/Eg7 for Chromosome Condensation in Mitotic Extract

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Abstract. Association of the condensin multiprotein complex with chromatin is required for chromosome condensation at mitosis. What regulates condensin targeting to chromatin is largely unknown. We previously showed that the nuclear A kinase–anchoring protein, AKAP95, is implicated in chromosome condensation. We demonstrate here that AKAP95 acts as a targeting protein for human chromosome-associated protein (hCAP)-D2/Eg7, a component of the human condensin complex, to chromosomes. In HeLa cell mitotic extract, AKAP95 redistributes from the nuclear matrix to chromatin. When association of AKAP95 with chromatin is prevented, the chromatin does not condense. Condensation is rescued by a recombinant AKAP95 peptide containing the 306 COOH-terminal amino acids of AKAP95. Recombinant AKAP95 binds chromatin and elicits recruitment of Eg7 to chromosomes in a concentration-dependent manner. Amount of Eg7 recruited correlates with extent of chromosome condensation: resolution into distinct chromosomes is obtained only when near-endogenous levels of Eg7 are recruited. Eg7 and AKAP95 immunofluorescently colocalize to the central region of methanol-fixed metaphase chromosomes. GST pull-down data also suggest that AKAP95 recruits several condensin subunits. The results implicate AKAP95 as a receptor that assists condensin targeting to chromosomes.

Key words: chromosome • condensin • AKAP • hCAP • mitosis

Introduction

The eukaryotic genome must compact and resolve into distinct chromosomes for proper segregation at mitosis. Chromosome condensation requires a family of highly conserved ATPases called structural maintenance of chromosome (SMC)[1] proteins (Hirano and Mitchison, 1994; Hirano et al., 1997). SMCs associate with non-SMC proteins in complexes, termed condensins, directly implicated in chromosome condensation (Strunnikov and Jessberger, 1999). The *Xenopus* 13S condensin complex consists of two SMC proteins (XCAP-C and -E) and three non-SMC elements (XCAP-D2/pEg7, -G, and -H; Hirano et al., 1997; Cubizolles et al., 1998). Condensins are targeted to chromosomes at mitosis and in *Xenopus* egg extracts. Mitosis-specific phosphorylation of non-SMC proteins has been implicated in their targeting to chromosomes (Hirano et al., 1997). However, as both interphase and mitotic forms of condensins bind DNA in a similar manner (Kimura et al., 1998), additional processes are likely to regulate chromosomal targeting of condensins (Hirano et al., 1997).

SMC homologues exist in a variety of organisms ranging from yeast to mammals (Strunnikov and Jessberger, 1999). Four SMC proteins have been identified in humans in the form of two distinct complexes (Schmiesing et al., 1998). The human chromosome-associated protein (hCAP)-C/hCAP-E complex associates with chromosomes at mitosis and is required for chromosome condensation. The second complex (hSMC1/hSMC3) is required for metaphase progression (Schmiesing et al., 1998). What regulates the targeting of hCAP to chromosomes is unknown. However, the different behaviors of the two complexes during the cell cycle suggests that they may play distinct roles in chromosome architecture.

Recent antibody-blocking and rescue experiments have identified a role of the A kinase–anchoring protein, AKAP95, in chromatin condensation and maintenance of

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[1]*Abbreviations used in this paper:* AKAP, A kinase–anchoring protein; hCAP, human chromosome-associated protein; SMC, structural maintenance of chromosome.

condensed chromosomes during mitosis and in mitotic extract (Collas et al., 1999). The former process is independent of A kinase activity, however, the latter requires cAMP signalling via A kinase. Immunoprecipitations from mitotic chromatin revealed that AKAP95 resides in a complex with hCAP-D2/Eg7, the human homologue of XCAP-D2/pEg7 (Cubizolles et al., 1998; Collas et al., 1999). Immunoblocking of AKAP95 was also shown to inhibit targeting of Eg7 to chromatin, suggesting the involvement of AKAP95 in this process (Collas et al., 1999).

We demonstrate here that chromatin-bound AKAP95 acts as a targeting protein for hCAP-D2/Eg7 in a mitotic extract. The results suggest that AKAP95 provides an additional level of regulation for the association of the condensin complex with chromosomes.

Materials and Methods

Antibodies and Peptides

Affinity-purified polyclonal antibodies against AKAP95 were from Upstate Biotechnology (Coghlan et al., 1994). Monoclonal antibodies directed against the last 306 amino acids of human AKAP95 (Collas et al., 1999) and against the nuclear matrix protein NuMA were from Transduction Laboratories. The GST-AKAP95 fragment containing amino acids 387-692 of human AKAP95 (GST-AKAP95Δ1-386) was described previously (Eide et al., 1998). Rabbit affinity-purified anti-human Eg7 polyclonal antibodies were produced against a peptide comprising the last 15 amino acids of Eg7 (KTTPILRASARRHRS).

Nuclei, Nuclear Matrices and Chromatin

Interphase HeLa nuclei were isolated from confluent cells as described (Collas et al., 1999). For immunoblocking experiments, anti-NuMA antibodies or nonimmune mouse IgGs were introduced into purified nuclei (Collas et al., 1999). In short, HeLa nuclei were mildly permeabilized with 0.75 μg/ml lysolecithin for 15 min. After quenching excess lysolecithin with BSA, nuclei were washed and incubated at 2,000 nuclei/μl with anti-NuMA antibodies (1:40 dilution) or nonimmune IgGs. After 1 h on ice nuclei were washed through a sucrose cushion and antibody introduction into nuclei was verified by immunofluorescence (data not shown).

High salt-extracted nuclear matrices were prepared from purified nuclei essentially as described (Reyes et al., 1997). In brief, chromatin was digested with DNase I and RNase A in Triton X-100–containing buffer. Ammonium sulfate was added to 250 mM and after 5 min at 4°C samples were sedimented. The pellet was extracted with 2 M NaCl for 5 min at 4°C to remove all DNA and histones (Reyes et al., 1997). After sedimentation, the pellet constituted the nuclear matrix fraction. To isolate solubilized chromatin, nuclei or chromatin masses were digested with 5 U micrococcal nuclease in the presence of 1% Triton X-100 for 5 min at 37°C (Collas et al., 1999). Digestion was terminated by adding EDTA to 5 mM and the mixture was sedimented at 10,000 *g*. The supernatant (S1) was collected and the pellet was incubated in 2 mM EDTA. After sedimentation, the supernatant (S2) was combined with S1 to yield a soluble chromatin fraction. Proteins were precipitated with trichloroacetic acid and dissolved in SDS sample buffer.

Metaphase Chromosome Spreads

Metaphase chromosome spreads were prepared from HeLa cells synchronized with Colcemid as described previously (Minc et al., 1999). Cells were swollen in 10 mM Hepes-NaOH, pH 8, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 30 mM glycerol for 10 min on ice and centrifuged onto slides. Samples were washed and extracted in 10 mM Tris-HCl, pH 8, 120 mM KCl, 20 mM NaCl, and 0.5 mM EDTA for 5 min at 4°C before immunofluorescence analysis.

Mitotic Extracts and Chromatin Condensation Assay

HeLa cells synchronized in mitosis with 1 μM nocodazole were homogenized by sonication in lysis buffer, and the lysate cleared at 10,000 *g* for 10 min and at 200,000 *g* for 3 h to produce a mitotic cytosolic extract (Collas et al., 1999). Interphase extracts were prepared from unsynchronized cells as above except that EDTA was omitted from the lysis buffer.

Nuclear breakdown and chromatin condensation were carried out at 30°C for up to 2 h in mitotic extract containing an ATP generating system (Collas et al., 1999). Condensation was characterized by compaction of the chromatin and resolution into distinct chromosomes, as judged by DNA staining with Hoechst 33342 and phase contrast microscopy.

Immunological Procedures and GST Pull-down

Immunofluorescence and immunoblotting were performed as described earlier (Collas et al., 1999) except that cells or chromosomes were fixed with –20°C methanol for immunofluorescence. Photographs were prepared using the Aldus Photostyler software. For biochemical analyses nuclei, chromatin or matrices were purified from the extract by sedimentation at 1,000 *g* through 1 M sucrose.

For GST pull-downs, chromatin essentially devoid of endogenous AKAP95 (AKAP95-free chromatin; see Results) was incubated for 2 h in mitotic extract containing 500 ng/ml GST-AKAP95Δ1-386 or 500 ng/ml GST as a control. At the end of incubation, the chromatin was sedimented through 1 M sucrose and solubilized in 500 μl TKM buffer (50 mM Tris, pH 7.5, 25 mM KCl, and 5 mM $MgCl_2$) containing 5 U micrococcal nuclease. After 45 min at room temperature, insoluble material was removed by sedimentation, and 50 μl of a 50% slurry of glutathione-agarose beads was added to the soluble chromatin supernatant. After 2 h at 4°C, beads were sedimented, washed three times in TKM buffer, and proteins were eluted in boiling SDS sample buffer.

Results

AKAP95 Redistributes from the Nuclear Matrix to Chromatin upon Nuclear Disassembly in Mitotic Extract

We have previously reported that AKAP95 primarily cofractionates with detergent-, high salt-, DNase-, and RNase-resistant matrices of purified HeLa cell nuclei (Collas et al., 1999). At mitosis, AKAP95 associates with chromosomes but also exists as a minor soluble pool, suggesting that association of AKAP95 with chromatin is cell cycle regulated.

Redistribution of AKAP95 from the nuclear matrix to chromatin was demonstrated upon nuclear disassembly in a cell-free extract derived from mitotic HeLa cells. The extract supports disassembly of purified interphase HeLa nuclei, including nuclear envelope breakdown and chromatin condensation (Fig. 1 a). Western blotting analysis of nuclear matrix and chromatin fractions prepared at successive stages of nuclear disassembly indicated that AKAP95 was redistributed from the matrix to the chromatin within 30 min (Fig. 1 b). Release of AKAP95 from the nuclear matrix was further illustrated by solubilization of AKAP95 upon incubation of purified matrices for 1 h in mitotic extract, but not in interphase extract (Fig. 1 c). This assay demonstrates the redistribution of AKAP95 from the nuclear matrix to chromatin upon mitotic nuclear disassembly.

Association of AKAP95 with Chromatin Is a Prerequisite for Chromosome Condensation

To investigate the functional significance of the association of AKAP95 with chromatin during nuclear disassembly, we produced chromatin that was essentially devoid of endogenous AKAP95. To this end, monoclonal antibodies against the nuclear matrix protein NuMA, or control preimmune mouse IgGs were introduced into purified nuclei

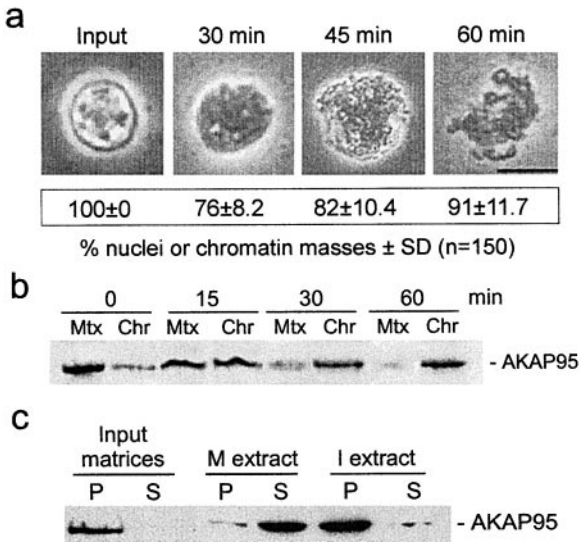

Figure 1. Dissociation of AKAP95 from the nuclear matrix upon nuclear breakdown in mitotic extract. (a) Purified HeLa nuclei were disassembled in mitotic HeLa cell extract and nuclear envelope breakdown and chromatin condensation were monitored by phase contrast microscopy. Proportions of nuclei or chromatin masses exhibiting the morphology shown are indicated (mean of three replicates). (b) Redistribution of AKAP95 was examined by immunoblotting of nuclear matrix (Mtx) and chromatin (Chr) fractions prepared at indicated time points of nuclear disassembly. (c) Nuclear matrices were prepared from HeLa nuclei (Input matrices) and exposed to mitotic (M extract) or interphase (I extract) extract for 1 h. Resulting matrices were sedimented and AKAP95 detected by immunoblotting in pellet (P) and supernatant (S) fractions. Bar, 10 μm.

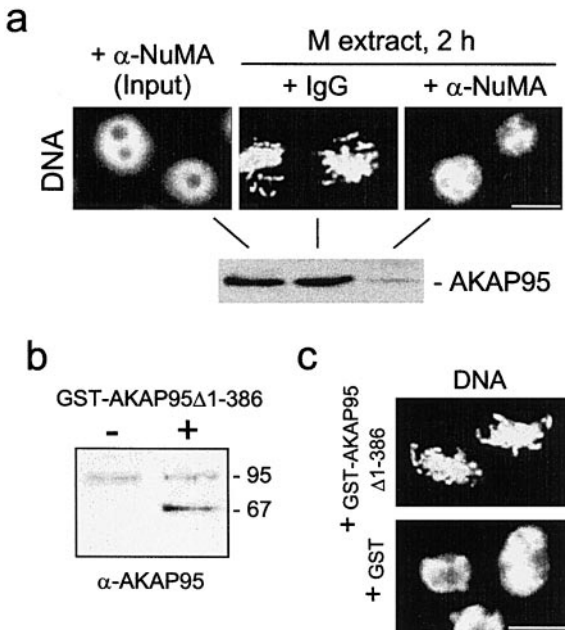

Figure 2. Association of AKAP95 with chromatin is required for chromosome condensation. (a) Production of chromatin mostly devoid of AKAP95. Purified HeLa nuclei were loaded with either anti-NuMA antibodies (+α-NuMA, Input) or preimmune mouse IgGs and exposed to mitotic extract for 2 h (M extract, 2 h). Chromatin morphology was examined after DNA staining with Hoechst 33342 (DNA). Chromatin was also sedimented through sucrose and immunoblotted using AKAP95 antibodies. Note the lack of condensation of chromatin devoid of AKAP95. (b) AKAP95-free chromatin was produced as in a and exposed for 1.5 h to mitotic extract containing 500 ng/ml GST (−) or GST-AKAP95Δ1-386 (+). GST-AKAP95Δ1-386 binding to chromatin was assessed on anti-AKAP95 immunoblot of purified chromatin at the end of incubation. The 95-kD band represents remaining endogenous AKAP95 while the 67-kD band represents GST-AKAP95Δ1-386. (c) Morphology of AKAP95-free chromatin was visualized by DNA staining with Hoechst 33342 after exposure to mitotic extract containing 500 ng/ml GST-AKAP95Δ1-386 or GST. Bars, 10 μm.

(Collas et al., 1999). These nuclei (Fig. 2 a, Input) were exposed to the mitotic extract for 2 h to evaluate chromatin condensation. Preimmune IgGs had no effect on chromatin condensation and association of AKAP95 with chromatin, as judged by DNA staining and Western blotting (Fig. 2 a, +IgG). Anti-NuMA antibodies, however, inhibited chromatin condensation, and AKAP95 was virtually absent from the chromatin, resulting in AKAP95-free chromatin (Fig. 2 a, +α-NuMA). In either situation, NuMA was solubilized (data not shown), suggesting that the lack of chromosome condensation resulted from the absence of targeting of AKAP95 to chromatin rather than from persistence of the nuclear matrix. The mechanism of inhibition of AKAP95 targeting to chromatin by anti-NuMA antibodies is under investigation.

A recombinant AKAP95 fragment comprising the COOH-terminal 306 amino acids of AKAP95 (GST-AKAP95Δ1-386) was capable of rescuing chromosome condensation. When AKAP95-free chromatin was incubated into mitotic extract containing 500 ng/ml GST-AKAP95Δ1-386, or as a control 500 ng/ml GST, we found that GST-AKAP95Δ1-386 readily bound chromatin, as shown by immunoblotting (Fig. 2 b, 67-kD band). The amount of GST-AKAP95Δ1-386 detected on blots of chromatin was similar to, or lower than, that of endogenous AKAP95 (data not shown), suggesting that levels of recombinant AKAP95 peptide bound to chromatin were near physiological. Remarkably, GST-AKAP95Δ1-386 also restored condensation of the chromatin into distinct chromosomes over 1.5 h while GST alone was ineffective (Fig. 2 c). Thus, GST-AKAP95Δ1-386 was sufficient to bind chromatin and restore chromosome condensation. We concluded that association of AKAP95 with chromatin was necessary for chromosome condensation.

AKAP95 Recruits hCAP-D2/Eg7, a Component of the Human Condensin Complex

We have previously shown that immunoblocking of AKAP95 in HeLa cell nuclei hinders chromatin condensation and correlates with the absence on chromatin of hCAP-D2/Eg7, the human homologue of *Xenopus* XCAP-D2/pEg7 (Cubizolles et al., 1998; Collas et al., 1999). Nonetheless, no evidence that AKAP95 was directly responsible for targeting Eg7 to chromatin was provided. To address this issue, we first examined the subcellular distribution of Eg7 during the HeLa cell cycle, in immunofluo-

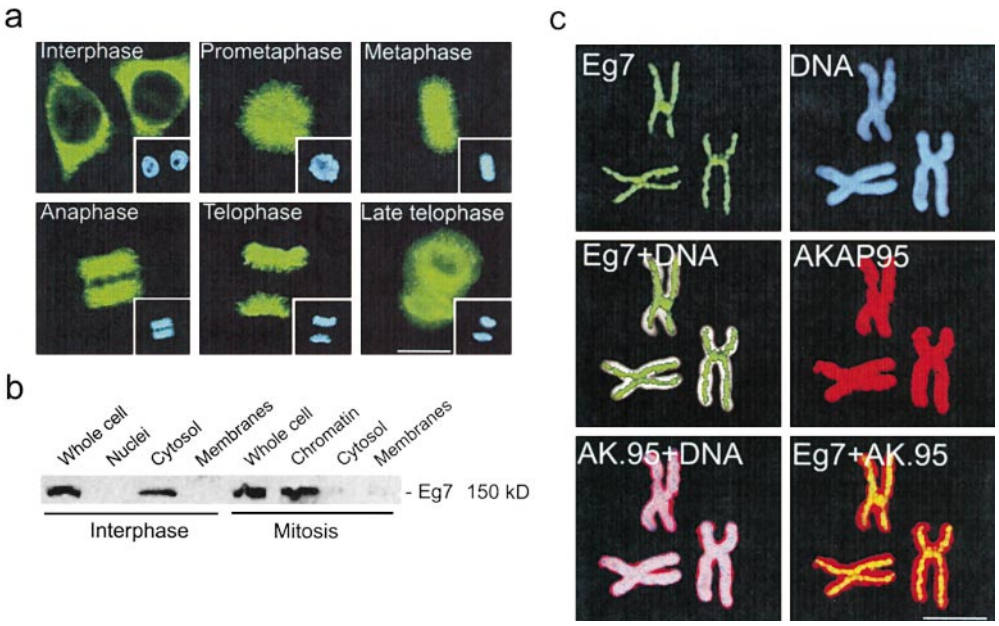

Figure 3. Subcellular distribution of hCAP-D2/Eg7 during the HeLa cell cycle. (a) Unsynchronized cells were fixed with methanol and distribution of Eg7 examined by immunofluorescence using affinity-purified anti-human Eg7 antibodies and FITC-conjugated secondary antibodies. DNA was stained with Hoechst 33342 (insets). (b) Interphase and mitotic cells were fractionated into nuclei or chromatin, cytosol, and cytoplasmic membranes, and each fraction was immunoblotted using anti-Eg7 antibodies. (c) Spreads of HeLa metaphase chromosomes were immunofluorescently labeled with anti-Eg7 and anti-AKAP95 antibodies. Anti-Eg7 antibodies decorate only the central core of chromosomes. DNA was stained with Hoechst. Bars: (a) 10 μm; (c) 5 μm.

rescence and cell fractionation studies using a newly developed affinity-purified polyclonal antibody against human Eg7. In interphase, Eg7 was cytoplasmic and identified in a soluble cytosolic (200,000-*g* supernatant) fraction (Fig. 3, a and b). As early as prometaphase and up to telophase, Eg7 was primarily restricted to the chromatin although a minor soluble fraction subsisted (Fig. 3, a and b). Examination of metaphase chromosome spreads showed that anti-Eg7 antibodies decorated the central core of chromosomes while anti-AKAP95 antibodies decorated the entire chromosomes and colocalized with DNA (Fig. 3 c). In late telophase, Eg7 labeling of the chromatin became weaker as the protein was also largely redistributed into the cytoplasm (Fig. 3 a), likely as a result of disassembly of the condensin complex from chromosomes (Kimura et al., 1998). These results indicate a cell cycle–dependent association of Eg7 with chromosomes, onto which it colocalizes with AKAP95.

Whether AKAP95 played a direct role in recruiting Eg7 to chromatin was determined by producing chromatin either devoid of AKAP95, or harboring GST-AKAP95Δ1-386 as described in the previous section. Control chromatin containing endogenous AKAP95 was obtained from nuclei condensed in mitotic extract. Chromatin contents in AKAP95 or GST-AKAP95Δ1-386 were verified on Western blot (Fig. 4 a, top). Remarkably, Eg7 was selectively recruited to chromatin harboring either endogenous or recombinant AKAP95 but was absent from AKAP95-free chromatin (Fig. 4 a, bottom). Note that traces of endogenous AKAP95 detectable on blot of chromatin were not sufficient to elicit Eg7 recruitment (Fig. 4 a). This AKAP95 probably originated from the minor chromatin-associated fraction of AKAP95 within interphase nuclei (Fig. 1 b, 0 min; Collas et al., 1999). As expected from our previous observations, this AKAP95-free chromatin did not condense (see Fig. 2 a). Additional evidence for a role of chromatin-bound AKAP95 in mobilizing Eg7 was provided by the recruitment of increasing amounts of Eg7 as the concentration of GST-AKAP95Δ1-386 added to the extract was raised (Fig. 4 b). Moreover, a pull-down of GST-AKAP95Δ1-386 with glutathione-agarose beads from chromatin condensed in mitotic extract containing 500 ng/ml GST-AKAP95Δ1-386 brought down Eg7, while no Eg7 was associated with GST alone (Fig. 4 c, Blot). Analysis of proteins of the GST pull-down complexes by Coomassie blue staining revealed several polypeptides, including major bands of 165, 150, 135, 100, and 67 kD (Fig. 4 c), which corresponded in size to those of hCAP-C, hCAP-D2/Eg7, hCAP-E, the putative human homologue of XCAP-H (100 kD) and GST-AKAP95Δ1-386, respectively. This raises the possibility that subunits of the human condensin complex were present.

Implications of AKAP95-mediated targeting of increasing amounts of Eg7 to chromatin were addressed by assessing the extent of chromosome condensation at each concentration of GST-AKAP95Δ1-386 used in the previous experiment. Clearly, higher degrees of condensation were consistently achieved with increasing concentrations of AKAP95 peptide (Fig. 4 d). Whereas no significant condensation occurred with 0–5 ng/ml peptide (compare with Fig. 2 c, +GST), some condensation was detected at 10–50 ng/ml. Chromosome compaction was only seen at 100–500 ng/ml peptide (Fig. 4 d). However, as emphasized in photograph enlargements (Fig. 4 d, bottom) resolution into distinct chromosomes only took place with ≥500 ng/ml AKAP95 peptide. At this level, chromosomes were resolved to a degree similar to that of control chromosomes harboring endogenous AKAP95 (Fig. 4 d, left). This sug-

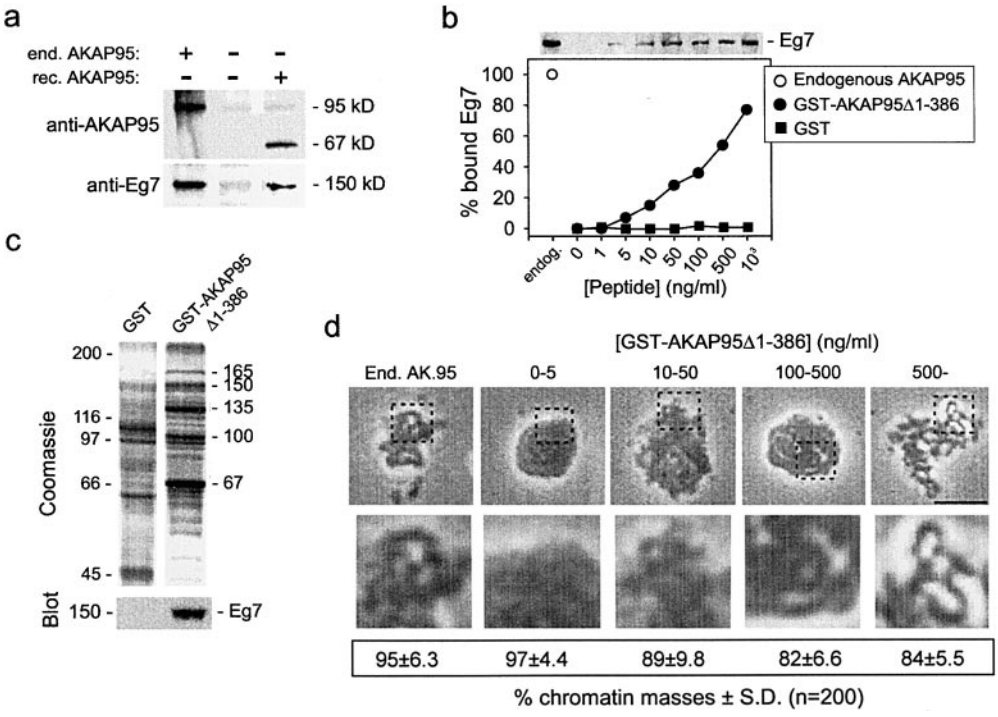

Figure 4. Association of AKAP95 with chromatin is necessary for recruitment of Eg7 to chromatin. Chromatin harboring endogenous AKAP95, no AKAP95, or GST-AKAP95Δ1-386 was produced as described in the text. (a) After a 1.5-h incubation in mitotic extracts, the chromatin was sedimented and immunoblotted using anti-AKAP95 antibodies (top). Recruitment of Eg7 to chromatin was assessed using anti-Eg7 antibodies (bottom). (b) AKAP95-free chromatin produced as in (a) was exposed for 1.5 h to mitotic extract containing increasing concentrations of GST (■) or GST-AKAP95Δ1-386 (●). Relative amounts of Eg7 recruited to chromatin were determined by immunoblotting and densitometric analysis of purified chromatin. Reference value (100%) was set using chromatin from untreated control nuclei harboring endogenous levels of AKAP95 (○). (c) Pull-down of GST-AKAP95Δ1-386 or control GST from chromatin condensed in extract containing 500 ng/ml GST-AKAP95Δ1-386. Pulled-down proteins were resolved by SDS-PAGE and stained with Coomassie blue (top) or immunoblotted using anti-Eg7 antibodies (bottom). (d) Phase contrast assessment of AKAP95-free chromatin after a 1.5-h exposure to indicated concentrations of GST-AKAP95Δ1-386 in mitotic extract. End. AK.95, endogenous AKAP95 (corresponding to control nuclei incubated in mitotic extract). Lower panels represent enlargements of areas framed in the upper panels. Proportions of chromatin masses displaying the morphology shown are indicated (mean of three replicates). Bar, 10 μm.

gests that chromosome condensation is limited by the amount of Eg7 or Eg7-associated polypeptides recruited to chromatin. Thus, in the absence of the majority of endogenous AKAP95, the recombinant AKAP95 fragment (a) binds chromatin, (b) recruits Eg7 in a concentration-dependent manner, and (c) elicits chromosome condensation. Resolution into distinct chromosomes takes place only when nearly endogenous levels of Eg7 are recruited. We conclude from these results that AKAP95 acts as a targeting molecule for Eg7, alone or as part of the condensin complex, to chromatin.

Discussion

Association of condensins with chromosomes is mitosis-specific. In *Xenopus*, all five 13S condensin subunits are found on chromosomes in mitotic extract (Hirano et al., 1997). In human somatic cells, hCAP-C/hCAP-E (Schmiesing et al., 1998) and hCAP-D2/Eg7 (this paper) are also targeted to chromosomes, while the hSMC1/hSMC3 complex appears cytoplasmic (Schmiesing et al., 1998). What regulates condensin targeting to chromosomes is largely unknown. Mitosis-specific phosphorylation of XCAP-D2/pEg7, -G, and -H correlates with their targeting to chromatin, leading to the attractive hypothesis that targeting of condensins to chromosomes may be regulated by phosphorylation (Hirano et al., 1997).

Our results provide strong evidence for an additional regulatory mechanism of condensin association with chromosomes. (a) Depleting chromatin of AKAP95 before nuclear disassembly in mitotic extract prevents chromatin condensation; (b) a recombinant AKAP95 fragment is capable of binding chromatin; (c) the fragment elicits recruitment of Eg7 to chromatin in a concentration-dependent manner; (d) a GST pull-down of recombinant AKAP95 brings down a complex containing Eg7; (e) recombinant AKAP95 induces chromosome condensation to an extent proportional to the concentration of peptide added and to the amount of Eg7 recruited to chromatin. We have previously shown that antibody blocking of AKAP95 in the interphase nucleus also blocked chromatin condensation and inhibited Eg7 recruitment to chromatin (Collas et al., 1999). Our present data extend this finding by showing that the 306 COOH-terminal residues of AKAP95 are sufficient to recruit Eg7 in a multiprotein complex, in a dose-dependent manner. Whether AKAP95 directly recruits Eg7 and condensins to chromosomes, or alternatively, whether the AKAP configures chromosomes to prepare them for condensin binding remains at present open. The latter hypothesis is suggested by the apparent association of AKAP95 with the entire chromosome, whereas Eg7 binds to the central core as judged by immunofluorescence. Nevertheless, our data argue for a relationship between the amount of Eg7 associated

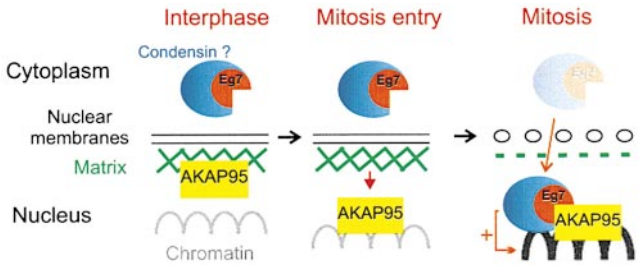

Figure 5. A model for AKAP95-mediated recruitment of Eg7 and condensins to chromatin at mitosis. In interphase, Eg7 is cytoplasmic and presumably associated with other subunits of the condensin complex, whereas AKAP95 resides primarily in the nuclear matrix. Upon mitosis entry, AKAP95 is readily released from the nuclear matrix and associates with chromatin. As nuclear membranes break down and the nuclear matrix solubilizes at mitosis, AKAP95 recruits Eg7/condensins to chromatin for chromosome condensation.

with chromatin, and the degree of chromosome resolution achieved during the condensation process.

Whether the entire condensin complex is recruited to chromosomes or whether individual subunits are targeted individually has not been, to our knowledge, specifically addressed. *Xenopus* egg extracts contain no free XCAP-C, although little XCAP-E is detected, such that condensins exist as complexes (Hirano et al., 1997; Cubizolles et al., 1998). Similarly, human SMCs have been isolated in the form of multimers (Schmiesing et al., 1998), so it appears that condensin subunits associate with one another even when not bound to chromosomes. Nevertheless, a clear answer to this issue awaits further investigations.

We propose a model for AKAP95-mediated recruitment of condensins to chromatin and chromosome condensation (Fig. 5). In interphase, AKAP95 resides in a complex within the nuclear matrix, while Eg7, perhaps in association with other condensin subunits, is restricted to the cytoplasm. Upon entry into mitosis, AKAP95 is released from the matrix and associates with chromatin. Subsequent disassembly of the nuclear envelope allows the recruitment of Eg7/condensin to chromatin for chromosome condensation. Our results implicate AKAP95 as a receptor protein that assists condensin targeting to chromosomes.

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