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Characterization and dynamic analysis of *Arabidopsis* condensin subunits, AtCAP-H and AtCAP-H2

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Abstract Condensin complexes are thought to play essential roles in mitotic chromosome assembly and segregation in eukaryotes. To date, two condensin complexes (condensin I and II) have been identified. Both complexes contain two structural maintenance of chromosome (SMC) subunits and three non-SMC subunits. In plants, little is known about the localization and function of all the condensin subunits. Here, we report the analyses on the localization of a non-SMC subunit of *Arabidopsis* condensin I and II, AtCAP-H, and AtCAP-H2, respectively. Our study indicated that localization of AtCAP-H and AtCAP-H2 is dynamically changed through the mitotic cell cycle using GFP-tagged AtCAP-H and AtCAP-H2 in tobacco cultured cells. They are localized at mitotic chromosomes from prometaphase to telophase. However, their localization in interphase is quite different. AtCAP-H was mainly found in the cytoplasm whereas AtCAP-H2 was mainly found in a nucleolus. It is revealed using GFP-tagged deletion mutants of AtCAP-H that the kleisin- γ middle domain (GM domain) is a unique domain only in AtCAP-H, responsible for chromosomal localization. We propose that the GM domain of CAP-H is essential for its chromosomal localization at mitosis and thus proper function of CAP-H. Differences in localization of AtCAP-H and AtCAP-H2 at interphase also suggest their functional differentiation.

Keywords *Arabidopsis thaliana* · Tobacco BY-2 · Chromosome · Condensin · GFP · Dynamic analysis

Abbreviations CAP: Chromosome Associate Protein · GFP: Green fluorescent protein · GM: Kleisin- γ middle · PEG: Polyethylene glycol · SMC: Structural maintenance of chromosome

Introduction

Proper chromatin condensation is essential for the normal segregation of chromatids during cell division. A condensin complex plays an essential role in mitotic chromosome assembly and segregation in eukaryotes (reviewed by Hearing and Nasmyth 2003). Condensin was identified first in *Xenopus* egg extract (Hirano et al. 1997). The condensin complex is composed of five subunits. These five proteins are conserved among eukaryotes (Sutani et al. 1999; Freeman et al. 2000; Schmiseing et al. 2000; Kimura et al. 2001). The two core subunits (CAP-E/SMC2 and CAP-C/SMC4) belong to SMC (structural maintenance of chromosomes) family protein. Two of the other three non-SMC subunits (CAP-D2 and CAP-G) share a highly degenerated repeating motif known as a HEAT repeat (Neuwald and Hirano 2000), and the rest of the three non-SMC proteins is CAP-H/kleisin- γ that belongs to a kleisin superfamily (Schleiffer et al. 2003). Immunodepletion or antibody blocking of condensin subunits for *Xenopus* egg extracts has shown that condensin subunits were essential for chromosome condensation and segregation in vitro (Hirano and Mitchison 1994; Hirano et al. 1997; Kimura and Hirano 2000; Wignall et al. 2003). Genetic studies in yeasts and metazoans have also revealed that condensin subunits are necessary for chromosome condensation and segregation in vivo (Bhat et al. 1996; Freeman et al. 2000; Sutani et al. 1999; Hagstrom et al. 2002).

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Recently, a second condensin complex (condensin II) has been identified in vertebrate cells (Ono et al. 2003; Yeong et al. 2003). The condensin II has the same pair of SMC subunits with condensin I but contains a different set of non-SMC subunits (CAP-D3, CAP-G2, and CAP-H2). The CAP-D3 and CAP-G2 also contain the HEAT repeat and CAP-H2 belongs to the kleisin superfamily. The condensin II also localized on mitotic chromosomes. Depletion of the condensin I or II specific subunits produces a highly characteristic defect in the chromosome morphology of HeLa cells. The condensin I and II show alternative distributions along the axis of a chromosome (Ono et al. 2004) and condensin II and I associate with chromosomes in this order, so they might have different functions to mitotic chromosome assembly (Hirota et al. 2004).

Proteins of the kleisin superfamily are found in archaea, bacteria, and eukaryote (Schleiffer et al. 2003). An alignment of the N- and C-terminal domains of the kleisin superfamily shows almost complete identity of the hydrophobic pattern and some conservation of functional residues. In eukaryotes, these proteins are classified into three types (kleisin- α , - β , and - γ). Kleisin- α includes non-SMC subunits of cohesin, Scc1, and Rec8. CAP-H and CAP-H2 belong to kleisin- γ and kleisin- β , respectively.

Studies on yeast cohesin complex showed that the N-terminal domain of the kleisin- α subunit (Scc1) interacts with the head of the SMC3 subunit while the C-terminal domain interacts with the head of the SMC1 subunit (Arumugam et al. 2003; Gruber et al. 2003; Haering et al. 2004). The middle domain contains cleavage sites for a thiol protease, separase. The proteolytic cleavage of Scc1's middle domain triggers the metaphase-to-anaphase transition (Uhlmann et al. 2000). From the finding that the N- and C-terminal sequences of the kleisin- γ subunit have similar conserved domains with kleisin- α , kleisin- γ might associate with the head domains of an SMC heterodimer in the manner similar with association between kleisin- α and SMC (Schleiffer et al. 2003).

Little information on plant condensins has been obtained compared to the rapid progresses in molecular and biochemical understanding of chromosome condensation in animals and yeasts. A few examples are that developmental function of SMC2 subunits (AtCAP-E1 and AtCAP-E2) in *Arabidopsis thaliana* has been reported (Liu et al. 2002; Siddiqui et al. 2003). A database search shows that *A. thaliana* also has condensin I and II. Thus, it is reasonable to speculate that the condensins are widely spread in the plant kingdom.

In this paper, we report the characterization and dynamic analyses of *Arabidopsis* condensins non-SMC subunits, AtCAP-H and AtCAP-H2. This study has elucidated that the two condensins localized differently and thus they may diverge in their functions as well. It has also been shown that the conserved domain in the middle region of AtCAP-H is essential for its localization in mitotic chromosomes.

Materials and methods

Plant materials

Arabidopsis thaliana ecotype Columbia (Col-0) was used for isolation of AtCAP-H and AtCAP-H2. Plants were grown at 22°C under a 16-h light/8 h dark regime. *A. thaliana* cell line T87 was obtained from the RIKEN BioResource Center (Tsukuba, Japan). The T87 cells were maintained at 23°C under continuous light. *Nicotiana tabacum* suspension culture cell line, Bright Yellow 2 (BY-2), was used for the localization analysis using GFP fusion genes. The BY-2 cells were subcultured weekly, 50-fold diluted with Linsmaier and Skoog (LS) medium according to Nagata et al. (1992). The cell suspension was agitated on a rotary shaker at 130 rpm at 25°C in the dark.

RT-PCR analyses

Total RNA was extracted from *Arabidopsis* roots, stems, leaves, flower buds, flowers, and whole plants germinated after three days using TRI REAGENT (Molecular Research Center, Inc. Cincinnati, OH, USA). The mRNAs were isolated from the total RNA samples using PolyATtract mRNA Isolation Systems (Promega). The mRNA was used for the first strand cDNA synthesis using a poly dT primer.

The cDNA fragments of AtCAP-H and AtCAP-H2 were amplified using gene specific primers (5'-GCTGCTAAACAATTTAGGAGTCTATGG-3' and 5'-GCAGGTTGAACATCTGGACCTT-3' for AtCAP-H, 5'-TCACACCTGGATGCTCTTCTCGC-3' and 5'-CTACACAATCTAATTTGAGTAGTCACAAT-3' for AtCAP-H2). RT-PCR was also performed with *actin2* primers as a control (Ratcliffe et al. 2003).

Cloning of the *Arabidopsis* CAP-H and CAP-H2 genes

Cloning of AtCAP-H and AtCAP-H2 was performed as described by Sambrook et al. (1989). The coding sequences of AtCAP-H (At2g32590) and AtCAP-H2 (At3g16730) were amplified using gene-specific primers with the recombination site (*AtCAP-H*, 5'-AA-*AAAGCAGGCTCCATGGATGAATCCTTA*-ACTCC-AAACCC-3' and 5'-AGAAAGCTGGGTCTC-AGGC-AAGGTGTATTGTTAGATCATCC-3'; *AtCAP-H2*, 5'-AAAAAGCAGGCTCAATGACCAGTCACGGTG-GCGGG-3' and 5'-AGAAAGCTGGGTTTCACAA-TTTCACCAAGATTTTTTCGATTGAAGC-3'. The recombination sites are shown in italic). The amplified fragments were cloned into pDONE221 using an in vitro recombination system (Invitrogen). The nucleotide sequences were checked with a DNA sequencer (ABI prism 3100 Genetic analyzer, Applied Biosystems). Thereafter, cloned cDNAs were recombined into the binary vector,

a

AtCAP-H

MDES^{LT}PNPKQKPA^{STTT}TRIQAP^{TS}PF^{FL}GSND^{DR}LEREQ 40
 ARA^{AAAA}SR^{RR}SVIF^{FARG}SQ^{PET}ES^{DP}CF^{DK}Q^QIL^{EL}LFQ 80
 NC^{IK}LASE^{NK}IN^{QK}NT^{WEL}NL^{ID}HL^{CE}IL^{KV}ED^{EN}NT^{ET} 120
 FQ^{KAS}CTLE^{AG}V^{KI}YS^{MR}V^{DS}VH^{SE}AY^{KV}LG^{GI}TRAG^{HD} 160
 GGD^{HEDA}AGAVENAT^{NQ}KKQ^{PE}KKIS^{PL}STLE^{PS}FDAL^{NV} 200
 KKF^{DV}AF^{AV}DP^{LY}HQ^{TS}AQ^{FD}EGGAK^{GLL}LN^{LN}LV^{GV}GGCQ 240
 VL^{FD}SQ^{EI}PG^{KLV}S^{SANK}H^{DK}SE^{TID}LS^{FV}KE^{CV}EQ^{ML}N 280
 MR^{KK}DE^{IV}PS^{LRAI}IN^{QF}DE^{EN}QR^{PS}DT^{FS}CG^{QO}TT^{ES}FD 320
 IS^{HG}ND^{AS}YAD^{DE}GE^{YEN}FG^{TS}FD^{YEG}Q^{SG}D^{VDE}N^FGP^{NE} 360
 AE^{PI}YS^{NF}HE^{EVE}PAS^{LQ}DM^{SD}DR^{LEN}V^{DD}YL^{FL}SL^{GIS} 400
 SK^{QNS}W^{AG}PD^{HW}K^{YR}K^{TK}G^{PD}V^QPA^{SE}IK^{SS}PP^{AK}K^{TR}TK 440
 KQ^{AE}PE^{LD}FA^{KALE}EE^{MP}DI^{FAP}PK^{NPK}T^{LLL}PA^{SRT}PCQ 480
 TK^{LP}ED^{CH}YQ^{PEN}L^{IK}FL^{LL}PN^{VM}CL^{GRR}RR^{RK}NS^{GE}TS^{SR}Q 520
 QP^{DD}YEH^{GES}W^{GN}D^{NV}Y^{DDD}GG^{PF}DD^{NEN}D^QSD^{AED}T^NTL 560
 IS^QPR^{QV}NK^{ID}V^QDK^{ASK}Q^{VD}V^QVL^{KE}T^WEC^{LQ}ES^{HQ}P 600
 PI^QDE^{EH}Q^QEP^{PE}S^{RS}FK^{VLL}AS^{FP}DD^{CQ}AA^{ERT}Q^DIS^{PH} 640
 LC^{FI}CL^{LH}LAN^{EHN}LS^{LIG}SQ^{NL}DD^{LT}IHLA* 671

**AtCAP-H2**

MT^{SH}GG^{GE}VR^{GER}IHT^{VQ}PER^{DL}VAN^{WE}VD^{LSE}K^{LEE}Y^{LL} 40
 K^{ICS}GE^{IT}G^{NEE}D^{GQ}IP^{VN}FA^{EAL}LL^{LQ}GS^{VQ}V^{YS}SK^{KVE}Y 80
 L^{YN}L^{VL}RT^{LE}FL^{SK}QR^{DQ}EQ^{SK}GT^{SN}ENE^{ASS}R^{QV}DE^{EE} 120
 ND^{LF}WN^{VDD}IP^{VDT}KN^{RLD}SS^{VGG}DT^{CP}SQ^{FV}K^{PP}AN^LV 160
 LE^{GD}CL^{DT}SG^{DG}GE^{LES}Y^{LL}ATTHLYRDF^{ILL}D^{PC}DA^{VAV} 200
 NE^{FL}GD^{NY}GG^{KGR}NS^{AHR}G^{SS}SV^{RKS}FS^{HSS}V^{GR}SG^{GS}S^{ARK}S 240
 SV^{GKN}Q^{GT}N^{VHL}SP^{IC}GN^{GP}ND^{QNC}D^{QGS}Q^{PP}V^{FED}ND^{HG} 280
 FD^{MD}NEY^{GG}AM^DFS^{DT}DA^{ED}DD^{PW}K^{PLN}PE^{PG}KL^{KV}K^{VP}F 320
 KK^{VK}IL^{KK}IG^{WS}IT^{KD}H^{MT}SM^{FPL}AR^{PNG}PI^{SSE}L^{EI}EW^K 360
 MH^{GC}ASK^{DE}Q^{AS}Q^{DI}PY^{YE}KL^{RE}ML^{VNG}N^QPC^{GAN}GN^{YN} 400
 DN^{DK}DN^{HDE}AN^{NG}D^{FHD}FE^{HD}GD^{DA}E^{HP}FM^{DE}D^VLN^{MND} 440
 GG^{AA}E^{FHN}Y^DGF^{ENG}ES^{NC}Q^{ES}LE^{DL}CR^{SH}LD^{LALL}ANI^{AK} 480
 SE^{KQ}TD^{LA}AR^{VS}T^{WK}Q^{KIE}QN^{LEE}Q^{EL}HP^{FD}I^QE^YGD^{RI} 520
 IN^{KL}T^{VE}ES^{GN}VE^{TFT}DL^{MKD}Q^{EK}HE^{VAR}AF^{SALL}Q^{LV}NN 560
 GD^{VD}LE^{KP}GNSTNE^{PMC}Y^{TAV}K^{PF}SV^{RLL}K^{VHN}R^{KNE}K^{RG} 600
 IH^{LP}Q^{KRA}K^{SPI}T^{KGS}HE^{SPP}PK^{RNT}CS^{VSS}Q^{TR}K^{VSL} 640
 K^{IS}K^{ING}V^{GV}RC^{TP}NS^{KRR}K^{RGR}S^{DD}V^{TE}V^{TE}V^{AS}IE^{KSL} 680
 GK^L* 683



b

<i>Arabidopsis thaliana</i>	WAGPDHWKYR
<i>Oryza sativa</i>	WAGPEHWKYR
<i>Homo sapiens</i>	WAGPDHWRF
<i>Mus musculus</i>	WAGPDHWRF
<i>Xenopus laevis</i>	WAGPEHWRF
<i>Drosophila melanogaster</i>	WAGPSHWKFK
<i>Shizosaccharomyces pombe</i>	WAGPEHWRIQ
<i>Saccharomyces cerevisiae</i>	WRGREHWKVR

pGWB6, with a recombination cassette for the expression of GFP-fused proteins under the control of a CaMV35S promoter. Deletion mutants were generated with PCR primers incorporating the deletion, and the deletion of each construct was confirmed by DNA sequencing.

◀

Fig. 1 Sequence analyses of *AtCAP-H* and *AtCAP-H2*. **a** The deduced amino acid sequences of *AtCAP-H* and *AtCAP-H2*. The N- and C-terminal conserved regions in kleisin superfamily are underlined. The conserved domain in the middle region of kleisin- γ , GM (kleisin- γ middle) domain is boxed. The structure of *AtCAP-H* and *AtCAP-H2* genes composed of 13 exons and 12 introns, and 11 exons and 10 introns, respectively. **b** The alignment of GM domains of CAP-H in eukaryote. The amino acid sequences of the GM domain were extracted from CAP-H of *Arabidopsis thaliana*, *Oryza sativa* (NP 918091), *Homo sapiens* (NP 056156), *Mus musculus* (NP 659067), *Xenopus laevis* (AAH56095), *Drosophila melanogaster* (AAB40125), *Shizosaccharomyces pombe* (NP 587811), and *Saccharomyces cerevisiae* (NP 009455) (Schleiffer et al. 2003; Ono et al. 2003)

Transformation of *Arabidopsis* protoplasts

Polyethylene glycol (PEG)-mediated *A. thaliana* protoplast transformation was according to the method described by Leon et al. (2002) with some modifications. The T87 cells were incubated in enzyme solution containing 1% (w/v) Cellulase Onozuka R-10 (Yakult Honsha, Tokyo, Japan), 0.25% (w/v) Macerozyme R-10 (Yakult Honsha), 8 mM CaCl₂, 0.5 M mannitol, pH 5.5 for 2 h at room temperature under gentle shaking. Protoplasts were collected by centrifugation for 5 min at 75 g, washed twice with W5 solution (154 mM NaCl, 125 mM CaCl₂, 5 mM KCl, and 5 mM glucose, pH 5.8), and resuspended in MaMg solution (0.4 M mannitol, 15 mM MgCl₂, and 5 mM MES pH 5.7). After incubation on ice for 30 min, protoplasts were adjusted to 1×10⁶ cells/ml with MaMg solution. A 100- μ l of PEG solution (40% (w/v) polyethylene glycol, 0.4 M mannitol, and 0.1 M Ca(NO₃)₂) was added for 100 μ l of plasmid-protoplasts solution and mixed gently. After incubation for 20 min at room temperature, 400 μ l of W5 solution was added and the PEG solution was removed by centrifugation for 5 min at 75 g. The protoplasts were resuspended in W5 solution and left at 23°C.

Transformation of BY-2 cells

Transformation was performed using BY-2 cells 2 days after subculture as described previously (Fujimoto et al. 2004). After 3-day incubation at 25°C, the inoculated BY-2 cells were transferred into liquid LS medium containing 500 μ g/ml carbenicillin and 10 μ g/ml hygromycin. The resulting transformed cell lines were maintained by 50-fold dilutions at weekly intervals as described above.

Fluorescence microscopy

Two days after subculture, BY-2 cells were used for the localization analysis. DNA in the cells were visualized with 0.25 μ g/ml of DAPI (4', 6-diamidino-2-phenylin-

dole) containing 0.01% Triton X-100. The cell images were obtained with a Zeiss Axioplan II fluorescence microscope and a cooled CCD camera (MicroMax, Roper Scientific Princeton Instruments, Trenton, NJ, USA). Further image processing and assembly were performed with the Adobe Photoshop software (Adobe System, Mountain View, CA, USA).

Results

Characterization of *Arabidopsis* AtCAP-H and AtCAP-H2 genes

Based on the nucleotide sequences of putative *AtCAP-H* (At2g32590) and *AtCAP-H2*(At3g16730), we designed the gene-specific primers to obtain the coding region of the genes and performed RT-PCR to amplify the cDNAs. Sequence analysis revealed that *AtCAP-H* contained a 2016-bp coding region with 13 exons in 3790-bp genomic sequence. *AtCAP-H2* contained a 2052-bp coding region with 11 exons in 3231-bp genomic sequence. The deduced AtCAP-H and AtCAP-H2 proteins have 671 amino acids and 683 amino acids, respectively. The amino acid sequences and gene structures are shown in Fig. 1a. The nucleotide sequences have been submitted to DDBJ under accession numbers AB193292 (*AtCAP-H*) and AB193291 (*AtCAP-H2*). AtCAP-H and AtCAP-H2 belong to the kleisin superfamily and also have the relatively conserved N- and C-terminal regions (Fig. 1a, underlined). In addition, AtCAP-H has the conserved domain among CAP-H in the middle region (Fig. 1a boxed, Fig. 1b). This domain was referred to as the kleisin- γ middle (GM) domain.

Then, we designed the genes-specific primers and analyzed the expression pattern of *AtCAP-H* and *AtCAP-H2* using semiquantitative reverse transcriptase-mediated (RT) PCR from *Arabidopsis* roots, stems, leaves, flower buds, flowers, and whole plants germinated after three days. *AtCAP-H* expressed at higher levels in flower bud and flower than the other organs. *AtCAP-H2* was not different in expression level among tissues examined (Fig. 2).

Subcellular localization of AtCAP-H and AtCAP-H2 in *Arabidopsis* cells

To investigate localization patterns of AtCAP-H and AtCAP-H2, transient expression of GFP-AtCAP-H and GFP-AtCAP-H2 were monitored in *A. thaliana* T87 cells. Approximately 0.01% of the cells showed GFP fluorescence in 1×10^5 cells, although about 1–10% of the cells showed GFP fluorescence in the case where only the GFP genes were transformed. As shown in Fig. 3, GFP-AtCAP-H was mainly localized in the cytoplasm while GFP-AtCAP-H2 was localized at the nucleus, especially in the nucleolus.

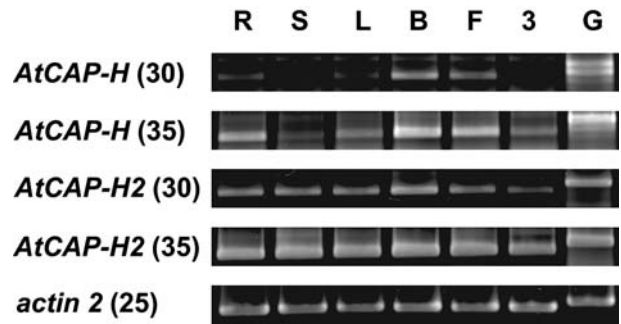


Fig. 2 Expression of *AtCAP-H* and *AtCAP-H2* in wild-type Columbia of *Arabidopsis thaliana*. Total RNA was extracted from root (L), stem (S), leaf (L), flower bud (B), flower (F), and whole plants three days after germination (3). Genomic DNA (G) was used as a control. Expression was monitored by RT-PCR. RT-PCR products were electrophoresed and stained with ethidium bromide. The PCR cycles are shown in the parentheses after the gene names

Dynamic localization of AtCAP-H in tobacco BY-2 cells

To investigate the dynamic localizations of AtCAP-H and AtCAP-H2, we transformed either *GFP-AtCAP-H* or *GFP-AtCAP-H2* into bright yellow-2 (BY-2) tobacco cells. BY-2 cultured cells are suitable materials for dynamic analyses during mitosis because the chromosome size is large and the cell cycle is short. The transformed cells were selected in the media containing hygromycin. In each transformation line, almost all cells show the same GFP fluorescence pattern. The growth rate and the morphology of each cell line were almost the same as the original BY-2 cells (data not shown).

Figure 4 shows the subcellular localization of GFP-AtCAP-H at different cell stages. In interphase, GFP signals were predominantly localized in the cytoplasm of the cells transformed with *GFP-AtCAP-H*. This localization pattern was the same as that in *Arabidopsis* T87 cells. GFP-AtCAP-H was not localized at mitotic chromosomes but localized at the cytoplasm until the

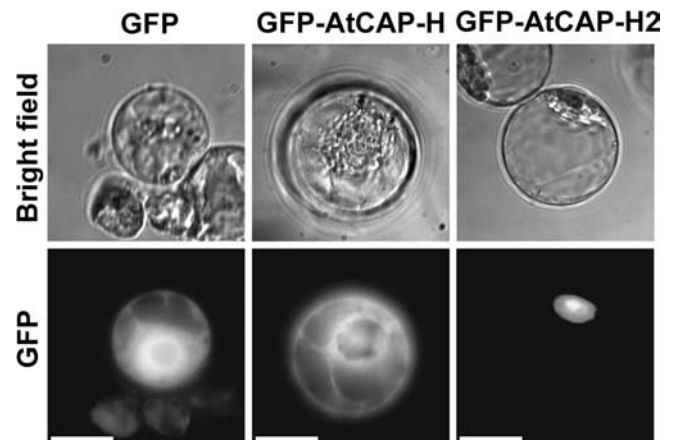


Fig. 3 Subcellular localization pattern of GFP-AtCAP-H and GFP-AtCAP-H2 in *Arabidopsis* protoplasts. The top column shows bright field images and the bottom shows GFP fluorescence. Scale bars 20 μ m

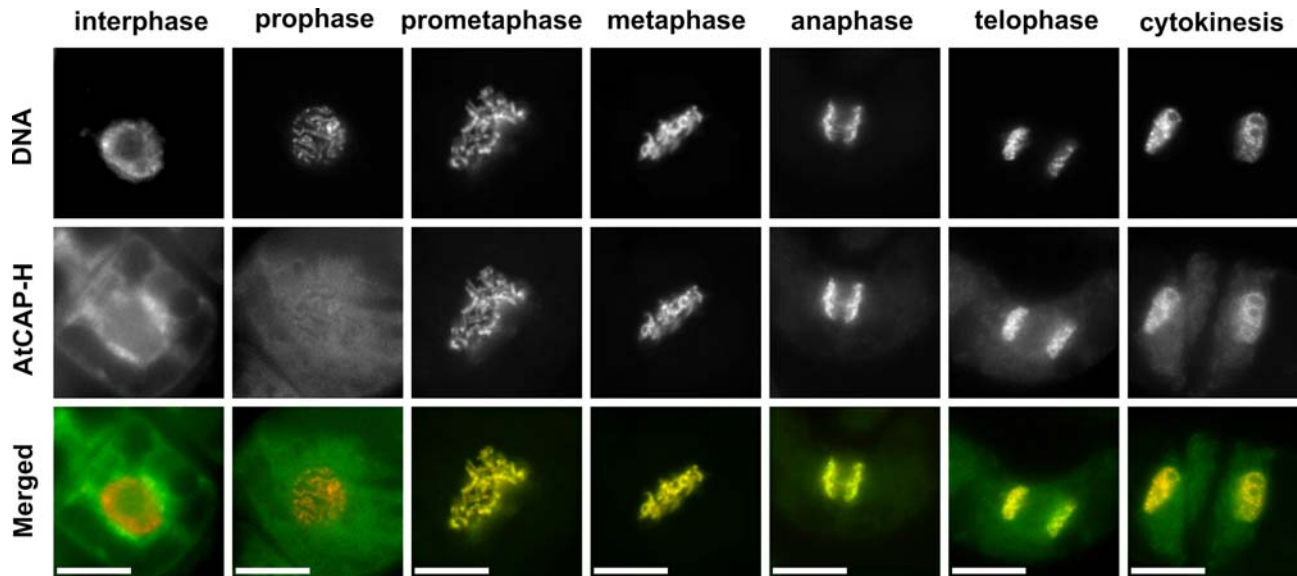


Fig. 4 Subcellular localization pattern of GFP-AtCAP-H in tobacco cultured cells. The top column shows DNA staining with DAPI and the middle shows GFP fluorescence. The *bottom column* shows merged images. GFP and DAPI are shown in *green* and *red*, respectively. Scale bars 20 μ m

end of prophase. In prometaphase, the signals were detected on the chromosomes. In metaphase, almost all the signals were moved to the chromosomes and the signal intensity was rapidly increased. After chromosome segregation, a few signals were diffused into the cytoplasm but the main signal remained on the chromosomes. The signals at the chromosomes were finally diffused to the cytoplasm after cytokinesis.

Dynamic localization of AtCAP-H2 in tobacco BY-2 cells

The dynamics in localization of GFP-AtCAP-H2 are shown in Fig. 5. In interphase, GFP signals were mainly detected in the nucleolus and slightly detected in the nucleoplasm. The GFP signals were also detected on the chromatin in interphase, suggesting that AtCAP-H2 interacts with interphase chromatin. This localization pattern was also the same as that in *Arabidopsis* T87 cells. The signals were localized in the nucleolus until the end of prophase. The signals were moved mainly to the entire chromosomes after the nucleolus disappeared. The signal intensity on the chromosomes was weaker than that of AtCAP-H. During the chromosome segregation, the signals were equally localized on both chromatids. When the nucleolus was formed in the nucleus, the signals appeared again in the nucleolus.

Domain responsible for chromosome localization

AtCAP-H has two relatively conserved N- and C-terminal regions among the proteins in the kleisin superfamily. Thus, AtCAP-H was structurally differentiated

into the three conserved regions such as the N-terminal region (N-region, 1–199 aa), the middle region (M-region, 200–520 aa) and the C-terminal region (C-region, 521–671 aa). In addition, among the kleisin- γ family, a highly conserved ten amino acid sequence (GM domain, 406–415 aa, WAGPDHWKYR) was found in AtCAP-H. To dissect which domains of AtCAP-H are involved in such chromosomal localization, AtCAP-H deletion mutants were fused with GFP and their localizations in transformed BY-2 cells were examined.

The GFP-AtCAP-H deleted with only 151 aa of the C-terminal region (GFP-NM) or deleted with 199 aa of the C-terminal region (GFP-MC) localized at most of the mitotic chromosomes but the localization is slightly loose (Fig. 6). Similar results were obtained for GFP-AtCAP-H with 321 aa of the middle region (GFP-M), although the signals were observed not only on the chromosomes but also in the cytoplasm. However, the GFP-AtCAP-H with the N-region or C-region showed no longer localization at mitotic chromosomes (GFP-N and GFP-C). Thus, the protein domains responsible for chromosomal localization would be at least in the M-region of AtCAP-H. Moreover, the deletion of the GM domain (Fig. 1) resulted in no localization of the GFP-AtCAP-H (GFP-GM del.) at mitotic chromosomes. The mutant proteins with the GM domain were localized on chromosomes in mitotic cells. Thus, the GM domain is essential for AtCAP-H to be localized on mitotic chromosomes.

Discussion

In this study, we found that the distributions of the two *Arabidopsis* condensin subunits, AtCAP-H and AtCAP-

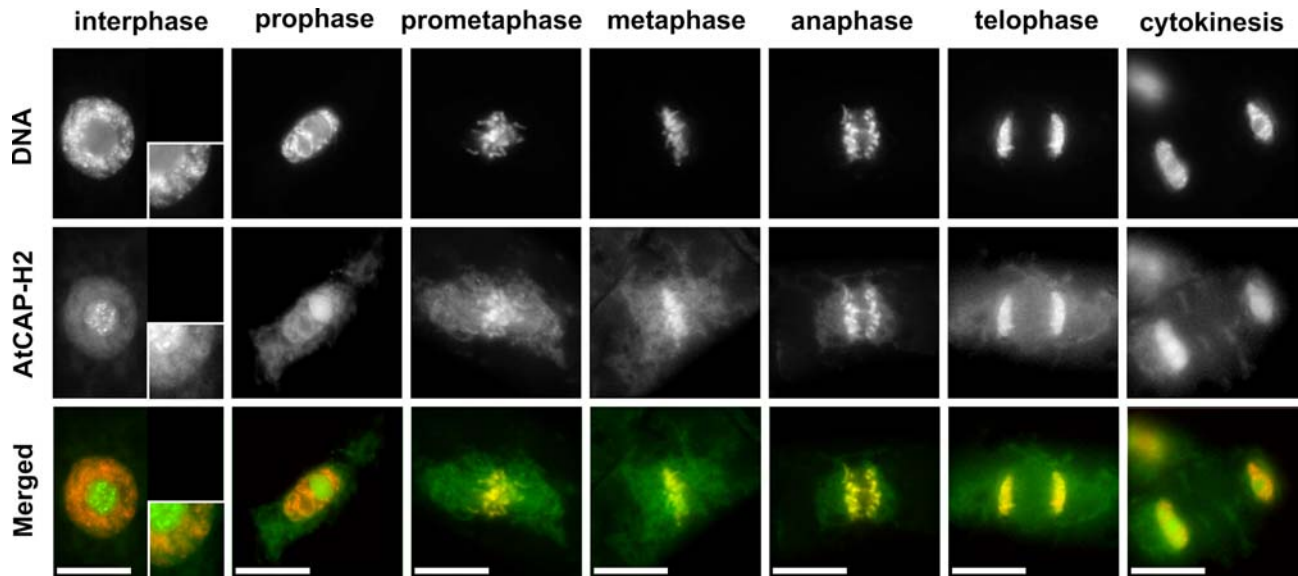


Fig. 5 Subcellular localization pattern of GFP-AtCAP-H2 in tobacco cultured cells. The lower right panels of the interphase images show the higher magnification of the same nucleus. The top

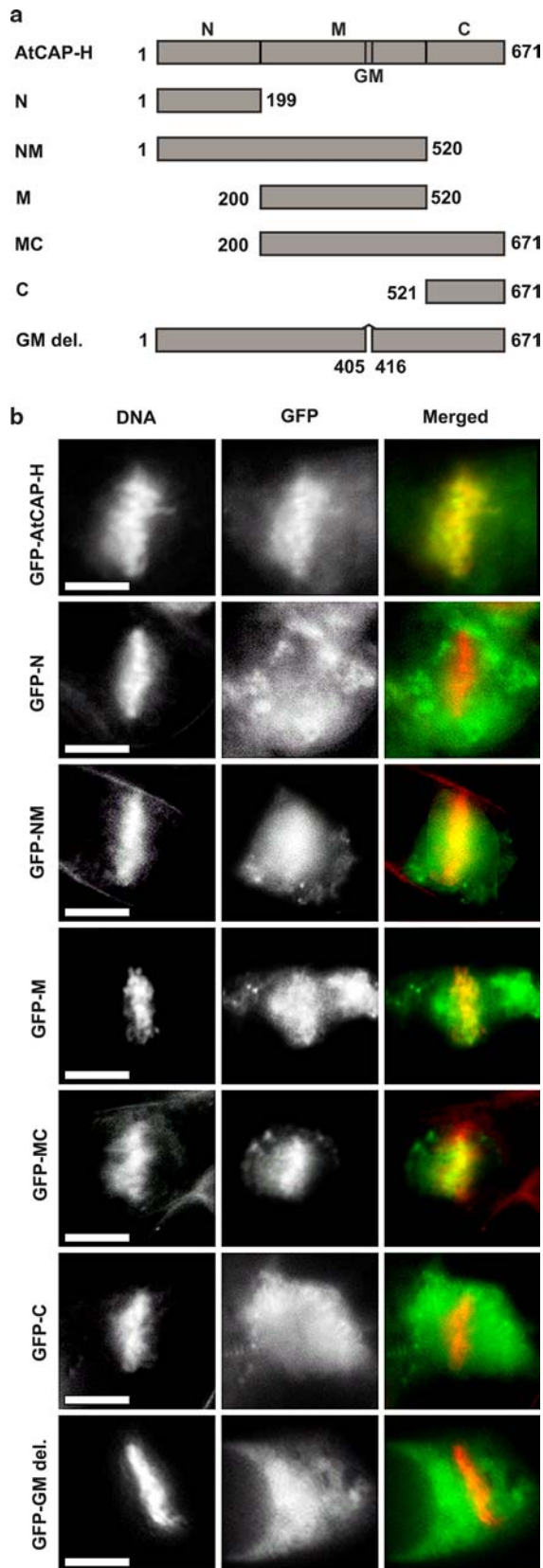
column shows DNA staining with DAPI and the middle shows GFP fluorescence. The bottom column shows merged images. GFP and DAPI are shown in *green* and *red*, respectively. *Scale bars* 20 μ m

H2, are different in localization except for prometaphase to telophase. Subcellular localization of the condensin subunits to chromosomes during mitosis has been well known among many organisms except for plants (Swedlow and Hirano 2003). In higher eukaryotes, some studies showed that the subunits are primarily localized in the interphase nucleus (e.g., XCAP-E, hCAP-E) (Hirano and Mitchison 1994; Shimizu et al. 1998) or in the nucleolus (e.g., hCAP-H, XCAP-E, XCAP-D2) (Cabello et al. 2001; Uzbekov et al. 2003), whereas others reported cytoplasmic localization of hCAP-E, hCAP-C, hCAP-D2, and *Drosophila* CAP-C (Saitoh et al. 1994; Schmiesing et al. 2000; Steffensen et al. 2001; Ball et al. 2002). Recently, the spatial and temporal distributions of condensin I and II have been shown to be differently regulated during the cell cycle in HeLa cells (Ono et al. 2004). In yeast cells, the fission yeast CAP-H subunit of the condensin complex, Cnd2, functions not only in essential mitotic chromosome condensation but also in DNA repair, S phase arrest and activation of a check-point kinase, Cds1 (Aono et al. 2002).

Dynamic analyses indicated that both AtCAP-H and AtCAP-H2 were localized on chromosomes from prometaphase to the cytokinesis. However, the most remarkable difference was observed in the distribution patterns in interphase. In interphase cells, AtCAP-H is localized in the cytoplasm, showing a striking contrast to the case of AtCAP-H2 that is localized in nuclei, especially in nucleoli at interphase. In vertebrate cells, hCAP-G (subunit of condensin I) is localized in the cytoplasm of the interphase cells. After nuclear envelope breakdown, hCAP-G is moved to the chromosome, and localized on the chromosome until cytokinesis (Ono et al. 2004; Hirota et al. 2004). Therefore, the localization patterns of AtCAP-H and human condensin I are similar to each other. In addition, hCAP-H2 (subunit of

condensin II) is localized in the nuclei at interphase. In prometaphase, hCAP-H2 is moved to chromosomes along with DNA, and co-localized with the DNA during the cell cycle (Ono et al. 2004; Hirota et al. 2004). The localization patterns of AtCAP-H2 and human condensin II are somewhat different, especially in interphase. In vertebrate cells, condensin II is thought to be a major form that contributes to the functional organization of the genome in interphase nucleus. Yeast condensin organizes the specialized topology of rDNA within a nucleolus and is important for silencing of the rDNA (Machin et al. 2004). Our results suggest that condensin II, rather than condensin I, mainly contributes to the organization of the genome, especially for the rDNA, in the interphase nucleus.

In the case of yeast cohesin, the connection between SMC1 and SMC3 heads linked by Scc1 is essential for the cohesion of sister chromatids. Thus, we assumed that N- and/or C-terminal regions of the AtCAP-H were important for its chromosomal localization because the triangular ring with the SMC1, SMC3, and Scc1 could trap two DNA molecules from the sister chromatid. However, we found that the M-region of AtCAP-H is sufficient for chromosomal localization, although there might be certain contribution of the other regions. Therefore, we hypothesized that the GM domain of 10 amino acid sequence in the M-region is essential for chromosomal localization. The further deletion experiment supported the hypothesis that the GM domain is essential for chromosomal localization of AtCAP-H. It is likely that kleisin- γ and SMC2-SMC4 heterodimers might associate and form a ring-like structure similar to these. The N- and C-terminal domains of Scc1 associate with the head domains of SMC1-SMC3 heterodimers and the heterodimers and kleisin- α form a ring-like structure (Arumugam et al. 2003; Gruber et al. 2003).



Our results demonstrate that CAP-H could partially localize on mitotic chromosomes regardless of the deletion of N- and/or C-terminal region. This fact shows



Fig. 6 Subcellular localization of GFP-AtCAP-H deletion mutant in mitosis of tobacco cultured cells. **a** A schematic diagram of each construct. The numbers at both ends indicate an amino acid number of AtCAP-H. **b** Subcellular localization pattern of AtCAP-H deletion fused to GFP. The left column shows DNA staining with DAPI and the middle shows GFP fluorescence. The right column shows merged images. GFP and DAPI are shown in *green* and *red*, respectively. In each transformation line, at least 10 mitotic cells were observed and most of them showed the same GFP fluorescent patterns. *Scale bars* 10 μ m

that the ring-like structure of SMC2 (CAP-E)-SMC4 (CAP-C) with CAP-H is not essential for localization of AtCAP-H on the chromosome. In addition, there is no GM domain in the other kleisin superfamily of subunit of cohesin or condensin II. Thus, the mechanism for chromosomal localization by the GM domain might be specific to CAP-H (kleisin- γ) family. The hinge conformations are remarkably different between the condensins and the cohesin SMC proteins (Anderson et al. 2002), indicating the possibility that the association manners of SMC proteins and kleisin superfamily would be different. The other possibility is that deletion of the GM domain causes unstabilization of interaction in the non-SMC subunits of the condensin I.

The condensin I and II complexes have been thought to be essential for chromosome condensation and segregation. Subcellular localization patterns of AtCAP-H and AtCAP-H2 suggest that plant condensins would have similar functions for the condensation and separation of the mitotic chromosomes in animals and yeasts. However, the localization pattern in interphase and the mechanism of chromosomal localization between them are quite different from previously reported results in other eukaryotic species. We suggest that the GM domain is a unique and conserved domain only in AtCAP-H and is essential for movement of AtCAP-H between cytoplasm and chromosomes at mitosis and thus the proper function of AtCAP-H. AtCAP-H moves from cytoplasm to chromosomes at the beginning of mitosis and moves back to cytoplasm after cytokinesis. On the other hand, AtCAP-H2 localizes at the nuclear or chromosomal regions throughout the cell cycle. The difference in localization may suggest the functional differentiation between condensin I and II in *Arabidopsis*.

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