**SMCI: An Essential Yeast Gene Encoding a Putative Head-Rod-Tail Protein Is Required for Nuclear Division and Defines a New Ubiquitous Protein Family**

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**Abstract.** The *smcl-1* mutant was identified initially as a mutant of *Saccharomyces cerevisiae* that had an elevated rate of minichromosome nondisjunction. We have cloned the wild-type *SMC1* gene. The sequence of the *SMC1* gene predicts that its product (Smclp) is a 141-kD protein, and antibodies against Smcl protein detect a protein with mobility of 165 kD. Analysis of the primary and putative secondary structure of Smclp suggests that it contains two central coiled-coil regions flanked by an amino-terminal nucleoside triphosphate (NTP)-binding head and a conserved carboxy-terminal tail. These analyses also indicate that Smclp is an evolutionary conserved protein and is a member of a new family of proteins ubiquitous among prokaryotes and eukaryotes. The *SMC1* gene is essential for viability. Several phenotypic characteristics of the mutant alleles of *smcl* gene indicate that its product is involved in some aspects of nuclear metabolism, most likely in chromosome segregation. The *smcl-1* and *smcl-2* mutants have a dramatic increase in mitotic loss of a chromosome fragment and chromosome III, respectively, but have no increase in mitotic recombination. Depletion of *SMC1* function in the ts mutant, *smcl-2*, causes a dramatic mitosis-related lethality. Smclp-depleted cells have a defect in nuclear division as evidenced by the absence of anaphase cells. This phenotype of the *smcl-2* mutant is not *RAD9* dependent. Based upon the facts that Smclp is a member of a ubiquitous family, and it is essential for yeast nuclear division, we propose that Smclp and Smclp-like proteins function in a fundamental aspect of prokaryotic and eukaryotic cell division.

The proper segregation of replicated chromosomes (sister chromatids) during mitosis in yeast requires a series of complex events, including specific metabolism of chromosomes and assembly and function of two complex molecular machines, the centromere and the spindle. By the beginning of mitosis, sister chromatids are paired and condensed. *Trans*-acting factors are assembled at specific centromeric DNA sequences on each chromosome to form the centromere while other factors are assembled with microtubules to form the mitotic spindle. The complex of centromeres bound to spindle microtubules mediatess various mitotic chromosome movements, including the migration of sister chromatids away from each other and towards the spindle poles during anaphase A (not detected in budding yeast). Separation of sister chromatids is increased further by elongation of the spindle (anaphase B). Chromosomes decondense, and the spindle disassembles. An important goal of researchers in the field has been to identify the different components responsible for each of these mitotic events and elucidate their molecular function.

In the budding yeast *Saccharomyces cerevisiae*, simple genetic assays were developed to monitor the fidelity of transmission of genetically marked natural and artificial chromosomes during cell division (e.g., Spencer et al., 1988). These assays exploit availability of yeast strains in which loss of a natural or artificial chromosome is not deleterious to the cell. Construction of artificial chromosomes has been feasible due to the identification of *cis*-acting yeast DNA sequences, including autonomous replication sequences (ARS) that nucleate the assembly of origins of replication (Stinchcomb et al., 1979), telomere sequences (TEL) that allow replication of the ends of linear chromosomes (Murray and Szostak, 1983a), and centromere sequences (CEN) that nucleate assembly of functional centromeres (Clarke and Carbon, 1980). Addition of CEN and ARS sequences to recombinant DNA vectors has generated

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1. Abbreviations used in this paper: ARS, autonomous replication sequences; cdc, cell-division cycle; CEN, centromere sequences; CFs, chromosome fragments; NTP, nucleoside triphosphate; ORF, open reading frame; TEL, telomere sequences.
small circular minichromosomes that are replicated and segregated with a fidelity as high as 99% per cell division (Murray and Szostak, 1983b; Hieter et al., 1985). CEN, ARS, and TEL sequences have also been combined with segments from yeast chromosome arms to generate chromosome fragments (CFs) (Vollrath et al., 1988). In general, short CFs (50–90 kb) are transmitted with a fidelity like circular minichromosomes, while long CFs exhibit a fidelity 50- to 100-fold greater (Murray et al., 1986; Palmer et al., 1990).

The assays for monitoring transmission of natural or artificial chromosomes have been used to isolate yeast mutants that lose natural or artificial chromosomes at an increased rate (Maine et al., 1984; Larionov et al., 1987, 1989; Spencer et al., 1988; McGrew et al., 1989; Hoyt et al., 1990). Since chromosome loss can occur either by improper chromosome replication or segregation, mutants loosing chromosomes at an increased rate could be defective in components required for either of these processes. However, frequent nondisjunction (2:0 segregation) of chromosomes in mitosis is a specific characteristic of a segregation defect, which could be used to discriminate between the two options mentioned. In previous studies we monitored nondisjunction levels in cell-division cycle (cyc) mutants (Palmer et al., 1990) and chromosome loss mutants (Larionov and Strunnikov, 1987; Kouprina et al., 1988) using sensitive assays following copy number of CFs or minichromosomes with dose-dependent markers. Among the many dozens of mutants screened, only a handful had an increased frequency of minichromosome nondisjunction (Kouprina et al., 1988; Palmer et al., 1990; Strunnikov, unpublished). The mutations in these few strains might correspond to genes encoding important segregation components.

One mutant from this subset, smcl-1 (stability of minichromosomes) (Larionov et al., 1985), had a rate of minichromosome nondisjunction at least 10 times greater than wild-type strains. Interestingly, however, this recessive mutation had no detectable effect on the transmission of chromosome III or on cell viability, indicating that it was probably a hypomorph. Given that the severe nondisjunction phenotype exemplified by the smcl-1 mutant was suggestive of a specific defect in the segregation mechanism, we decided to investigate further the SMCI gene and its product. Here we report the cloning of the SMCI gene, the structural analysis of the SMCI gene product, and the analysis of chromosome loss, cell viability, spindle morphology, and DNA distribution in Smcl+ and Smcl− cells. These studies reveal that the SMCI gene encodes a 165-kD protein that is essential for completion of chromosome segregation in yeast. Similarities between Smclp and putative proteins from other organisms indicate that the Smcl protein has structural and perhaps functional homologs in both prokaryotic cells and higher eukaryotes.

Materials and Methods

Strains

The Saccharomyces cerevisiae strains used in this work are listed in Table 1. All strains are congenic with the S288C background except the CF-containing strains. The original smcl-1 mutation was isolated in the strain GRF18 (ATCC 64667; American Type Culture Collection, Rockville, MD). The mutant strain r2-GRF18 (ATCC 64666) (Larionov et al., 1985) was used as the parent for all smcl-1 strains in this work. Isogenic pairs of haploid strains (smcl-1 and SMCI) were constructed by integrative transformation of the strain kAS112 with plasmids pAS135 (LEU2 and SMCI) and pAS136 (LEU2) targeted into unique Apal site downstream of smcl locus. The CR10 chromosomal fragment was introduced into smcl-1 strains by standard crosses with SMCI strains carrying corresponding chromosomal fragments (Palmer et al., 1990).

To construct congenic smcl ts strains, a SMCI diploid strain, AS153, was transformed with the Sac1–Spc1 fragment from plasmid pS160 (see below). The resulting diploid AS154 became heterozygous for the smcl-2 deletion. This diploid was transformed with integrative plasmid pAS167 harboring the smcl-2 allele. The plasmid had been cut at the StuI site in URA3 to target its integration to the URA3 locus. The AS154 transformants (AS172 and AS173) were sporulated, and haploid progeny were recovered that contained a deletion of the endogenous SMCI gene and the insertion of smcl-2 into the ura3 locus. These haploid strains were used to produce homozygous smcl-2 diploids AS175 and AS195. barl derivatives of the smcl-2 strains and the SMCI strain MAY589 were obtained via insertion of LEU2 gene into the chromosomal BAR1 locus, using a barl::LEU2 plasmid (MacKay et al., 1988) digested with XbaI. All the smcl-2 strains were produced and maintained as the SMCI plasmid-bearing strains.

### Table 1

| Strain     | Genotype                  | Source       |
|------------|---------------------------|--------------|
| MAY589     | MATa his3 leu2 ade2 ura3  | A. Hoyt      |
| MAY591     | MATa his3 leu2 lys2 ura3  | A. Hoyt      |
| YPH102     | MATa ade2 his3 leu2 lys2 ura3 | Ph. Hieter  |
| AS153      | MATa/MATa ade2/ADE2 his3 leu2 lys2/LYS2 Galt | This study   |
| AS203      | MATa/MATa ADE2/ade2 his3 LEU2/leu2 ura3 trpl/TPR1 | This study   |
| 5dAS98     | MATa ade2 his3 leu2 trpl ura3 smc1-1 | This study   |
| 1aAS112    | MATa ade2 leu2 ura3 smc1-1 | This study   |
| 1eAS112    | MATa ade2 leu2 ura3 smc1-1 | This study   |
| 1bAS148    | MATa ade2 leu2 ura3 [CF110: LEU2 ADE3] smc1-1 | This study   |
| 1bAS154    | MATa ade2 leu2 lys2 smcl-1Δ ura3 | This study   |
| 2bAS154    | MATa leu2 smcl-1Δ smc1-2 | This study   |
| AS248      | MATa/MATa leu2 ura3 smc1-Δ | This study   |
| 1bAS172    | MATa leu2 smcl-1Δ smc1-2 | This study   |
| 1aAS172    | MATa ade2 leu2 lys2 smcl-1Δ smc1-2 | This study   |
| AS175      | MATa ade2/ade2 leu3 smcl-1Δ | This study   |
| AS195      | MATa ade2/ade2 LEU2/leu2 smc1-Δ smc1-2 | This study   |
| AS241      | MATa ade2/ade2 LEU2/leu2 smc1-Δ smc1-2 | This study   |
| 3cAS196    | MATa ade2/ade2 LEU2/leu2 smc1-Δ smc1-2 | This study   |
| 4bAS196    | MATa rad9::LEU2 smc1-Δ smc1-2 | This study   |
| 3dAS196    | MATa leu2 trpl RAD9 smc1-Δ smc1-2 | This study   |
| 4aAS196    | MATa leu2 RAD9 smc1-Δ smc1-2 | This study   |

Plasmid Construction

The vectors used in this study were the pRS shuttle vectors (Sikorski and Hieter, 1989; Christianson et al., 1992) YCplac11 (Gietz and Sugino, 1985) was used as a source of the hybrid GAL10-CYC1 promoter. The two SMCI-bearing plasmids that complemented the smcl-1 mutation. They both contained 90-kb inserts with an identical pattern of restriction sites. One of these plasmids was chosen for further use and designated as pAS99. The integrative SMCI plasmid, pAS17, was made by inserting a 5.5-kb SpeI–SalI fragment of pAS99 into the corresponding sites of pRS316. The SMCI CEN plasmid, pAS128, was constructed by inserting the 5-kb SpeI–ScaI fragment of pAS127 into the corresponding polynucleotide linker sites of pRS415. The SMCI CEN plasmids, pAS410 and pAS141, were
constructed by inserting the SacI fragment of pAS127 into plasmids pTI15 and YCP1acl11, respectively. The integrative SMCI plasmid pAS135 was made by cloning the 4.5-kb MluI–BssHII fragment of pAS128 between the BssHII sites of pRS405, and its Smc1– derivative, pAS136, was made by deleting the HpaII site between BssHII sites of pRS405. Plasmids for disruption of the genomic copy of SMCI were made first by insertion of the HIS3 BamHII-EagII fragment into the corresponding sites of the SMCI gene. The resulting plasmid pAS125 (pUC19 backbone) can be cut with SacI and Spel to give a linear fragment used in the transformation for one-step gene replacement. pAS125 was modified further to delete the coding region of SMCI completely. For this purpose DNA sequences between MluI and BamHII sites were removed to give pAS160. Plasmids containing temperature-sensitive smc1 alleles were generated by hydroxylamine mutagenesis and plasmid shuffling essentially as described by (Silkorski and Boeke, 1991). After pAS128 DNA was treated with hydroxylamine, it was transformed into E. coli cells, and more than 106 transformants were collected and pooled. Plasmid DNA isolated from the pool was then used to transform the smc1Δ-2 strain bts1ΔS14 carrying the URA3-marked plasmid pAS140. Transformants that had lost pAS140 were selected by replica plating to plates containing 5-fluoroorotic acid. Resulting Ura−papillae were analyzed for temperature-sensitive growth on YEPD plates. pAS128ts plasmid DNA was isolated from the clones that acquired a ts phenotype, and then amplified in E. coli. The integrative plasmid pAS167 harboring smc1-2 allele was made via cloning the Xhol–NheI fragment of pAS128 into the pRS406 Xhol and XbaI sites.

A plasmid containing the original smc1-1 mutation was obtained via rescuing the marked smc1-1 allele from the genomic locus. The pAS233 plasmid (pRS406 containing the 3′ to SMCI Eagl–SacI fragment) was integrated into the Nhel site downstream of smc1 chromosomal locus (strain laciS12). To generate the plasmid DNA, the strain was then transformed, and isolated transformed yeast cells were digested with KpnI and ligated. After transformation of the E. coli cells, a plasmid carrying mutant smc1-1 gene (pAS242) was recovered. The plasmid complemented a deletion of the SMCI gene but not the smc1-1 mutation. Detailed description of a similar technique was published (Rothstein, 1991).

Plasmid pAS182 containing the SMCI gene driven by the galactose-inducible promoter was constructed in several steps. The backbone plasmid pAS78 was constructed by introducing GAL10-CYC1 promoter (the HindIII–BamHI piece of YCPG1) into pUC19. Then most of the SMCI coding sequence (the BamHI–BamHI fragment) was deleted from pAS127. The resulting plasmid pAS166 had only 918 bp from SMCI gene spanning the 5′ untranslated region and the beginning of the gene (upstream of BamHI site). An oligonucleotide (5′GATGTCGCAATGGGCTC1AG) containing the SMCI upstream sequence was synthesized and PCR amplification of the pAS166 fragment was made using the standard T3 primer as the second PCR primer. Resulting mixture of the reaction products was digested with BamHI and a 0.6-kb piece was cloned into pBluescript (ks+) to give pAS176. After the sequence analysis the fragment was used to assemble plasmid pAS182; both 0.6-kb BamHI fragment of pAS166 and 3-kb BamHI–SacI fragment of pAS127 were subsequently introduced into pAS176 opened with BamHI and SacI. All the junctions between the fragments were verified by sequencing. The plasmid pAS182 has both CYC1 and SMCI start codons, separated by four codons.

A derivative of the SMCI gene encoding an epitope tagged Smc1p under the control of GAL10-CYC1 promoter was constructed by cloning the Xhol–NheI fragment of the pAS182 into the Xhol–NheI site of pAS22, giving pAS197, pAS90 (Strunnikov, A., unpublished observation) and pAS122, giving pAS197, pAS97 (Strunnikov, A., unpublished observation) has six repeats of the myc tag (Roth and Gall, 1989) flanked by XhoI sites located in frame to each other. An NcoI–Xbal fragment of pAS197 that contained the myc tag was used to replace the NcoI–Xbal fragment of pAS128. Then the resulting plasmid pAS211, expressed the Smc1–myc protein under the control of SMCI promoter.

Construction of the plasmids for bacterial expression of different SMCI-derived polypeptides was made using a 5 × His expression vector pRSETa (Invitrogen, San Diego, CA). To express the carboxy-terminal part of Smc1p, the pAS185 plasmid was constructed by cloning the Spel–NheI region of pAS128 into the Nhel site of pRSETa. To express the amino-terminal part of Smc1p, a pAS182 fragment from the ATO-proximal BamHI site to EcoRI site of SMCI was purified. This fragment was inserted into the corresponding site of pRSETa to yield pAS198. Plasmids pAS185 and pAS198 together represent a whole SMCI coding region without a gap or an overlap.

**DNA Sequencing**

DNA sequences were determined by the dNTP termination method using an automated procedure involving differential fluorescent label. All sequencing reactions and running of the sequencing gels were performed in accordance with the ABI manual for automated sequencing on a 370A DNA sequencer (Applied Biosystems, Foster City, CA). To determine the primary nucleotide sequence of the SMCI gene, a series of nested deletions (Henikoff, 1984) of plasmid pAS128 were constructed using ExoHI and Mung bean nucleases. Both strands of the SMCI DNA were sequenced. Any ambiguity in the sequence was resolved utilizing 18-base primers complementary to the sequences adjacent to the region of ambiguity. To determine the sequence of the mutant genes, smc1-1 and smc1-2, primers complementary to the sense strand were made at 300–400-bp intervals along the open reading frame (ORF).

**Production and Purification of Recombinant Proteins**

To produce polypeptides coded by SMCI gene, a T7 expression system was used as recommended by Novagen for pET expression. Plasmids pAS185 and pAS198 were introduced into strain BL21(DE3) (Novagen, Madison, WI). Expression of the recombinant gene product was induced by 1 mM isopropyl/thio-β-D-galactoside (IPTG). After 4 h of induction at 30°C, cells were centrifuged, and the cell pellets were frozen at −70°C. After thawing on ice pellets, the cells were resuspended in TN buffer (10 mM Tris [pH 8.0], 0.2 M NaCl) containing lysozyme (0.2 mg/ml) and protease inhibitor cocktail and incubated at 4°C for 1 h. Then nine volumes of 8 M urea were added and the cell suspension was stirred at room temperature for 1 h. The lysate was cleared by spinning at 19K (1A-20 rotor; Beckman Instrs. Carlsbad, CA) for 20 min at 23°C, and loaded onto an affinity column pre-equilibrated with the identical buffer. As all the columns were based on the vector pRSETa (Invitrogen) containing the NH2-terminal stretch of six histidine residues, one-step affinity purification of the fusion polypeptides was done. Affinity column was prepared from Ni-agarose (Quagen Inc., Cleveland, OH), and handled as recommended by Quagen. All affinity chromatography steps were done at 4°C. All elution buffers contained 8 M urea and β-mercaptoethanol and were adjusted to pH 8.0, 6.4, 5.8, and 4.7. Protein was bound to the column at pH 8.0; the column was washed with 10-bed volumes of corresponding buffer, then bound proteins were eluted with step pH gradient (10-bed volumes/step). At pH 5.8 maximal elution of the recombinant proteins was detected, so for this step a minimal volume of the elution buffer was used. All fractions were collected by PAGE, blotted onto nylon membrane, and probed with polyclonal antibodies (Novagen, Inc.) to the T7-tag encoded by the pRSETa vector (upstream of the fusion site). Aliquots that contained the maximal concentration of recombinant protein were pooled and subjected to dialysis at 4°C against 150 mM NaCl, 40 mM potassium phosphate (pH 8.0), 1 mM DMSO, and 0.1% SDS. Under these conditions proteins expressed from pAS185 and pAS198 (PEPI85 and PEPI98) precipitated. The precipitates were separated from the copurified proteins by centrifugation at 40,000 g, and washed twice with dialysis buffer. After the completion of this step, the average yield for PEPI85 and PEPI98 was 7 and 1 mg/liter of culture, respectively. No significant expression was obtained for pAS208 that contained the entire coding sequence of SMCI. The authenticity of PEPI85 as a product of SMCI was confirmed by the NH2-terminal peptide sequencing.

**Generation of Smc1p Specific Antibodies and Immunodetection**

Polypeptide PEPI85 was used as an immunogen to generate Smc1p COOH terminus-specific polyclonal antibodies. PEPI85 antigen purified from bacteria cells was prepared in two different ways. One rabbit was injected with PEPI85 purified by elution from a polyacrylamide gel, another with the Ni-
agarose-bound PEP185. Corresponding antisera C180 and C181 had close specificity and anti-Smclp antibodies titer. For immunofluorescent staining C180 and C181 antisera were affinity-purified on CNBr agarose column (Pharmacia Diagnostics, Inc., Fairfield, NJ) with the coupled gel-purified PEP185. Detection of Smclp and Smcl-myc on the membrane after Western blotting was done using anti-rabbit and anti-mouse Vectastain-Peroxidase kit (Vector Labs., Inc.). Total yeast protein for western analysis was extracted according to a published procedure (Ohashi et al., 1982).

Cytological Methods

Yeast nuclear DNA was visualized by DAPI staining of the formaldehyde-fixed cells. Indirect immunofluorescence of cells was performed essentially as described (Kilmartin and Adams, 1984). Microtubule structures were detected with the mouse monoclonal antibody YOLI/34 (1:200) (Kilmartin et al., 1982), and goat anti–mouse antibodies conjugated to rhodamine (Cappel Labs., Cochrannie, PA). The Smcl-myc protein was detected with the mouse monoclonal anti-myc antibody 9E10 (Evan et al., 1985) and the goat anti-mouse antibodies conjugated to rhodamine. The Smclp was monitored with the affinity-purified anti-Smclp rabbit antibodies and the goat anti-rabbit rhodamine-conjugated antibodies (Cappel Labs.).

Results

Cloning of SMCI Gene by Complementation of the smcl-1 Mutation

A measure of the mitotic stability of a chromosome is the percentage of cells in a culture that retain that chromosome under selection. Consistent with a previous report (Larionov et al., 1985), we observed that the mitotic stability of circular minichromosomes was dramatically reduced by the smcl-1 mutation. For example, in the smcl-1 mutant strain (lcAS112), the stability of CEN plasmids YCp41 and pSB32 was 62 ± 5% and 55 ± 6%, respectively, compared with 93 ± 2% and 89 ± 3% in the isogenic SMCI strain (YPH102).

The instability of CEN plasmids like pSB32 in smcl-1 cells results in a marked reduction in the growth rate of smcl-1 colonies under selective conditions. This phenotype of smcl-1 was chosen as a basis for screening for DNA clones that complemented the mutation. Approximately 30,000 transformants of the smcl-1 haploid strain (5dAS98) were selected after transformation with a CEN-vector based library (Spencer et al., 1988). Most of the transformants grew slowly because their plasmids were mitotically unstable in the presence of the smcl-1 mutation. However, more than 500 transformants gave colonies at least twice the average size after 3 d incubation at 30°C. These fast-growing transformants may have arisen because they contained plasmids with greater mitotic stability, in particular plasmids harboring genes that suppress or complement the smcl-1 mutation. In subsequent quantitative analyses, the plasmids in only two transformants reproducibly showed a mitotic stability of greater than 95%. When plasmid DNA was rescued from these transformants into E. coli, it became apparent that the transformants contained an identical plasmid (pAS99, Fig. 1) with a 9-kb insertion. When pAS99 was reintroduced into the smcl-1 strain it showed high mitotic stability. Furthermore, the mitotic stability of other CEN-based plasmids in smcl-1 cells reached wild-type levels in the presence of pAS99 (data not shown). These results demonstrated that pAS99 contained a DNA sequence that could complement the instability of CEN-based plasmids in smcl-1 cells.

To show that we cloned the SMCI gene and not an extragenic suppressor, the plasmid pAS103 was used for integrative transformation of Smcl+ haploid strain YPH102. This transformation targeted the URA3 marker to the chromosome locus corresponding to the genomic DNA fragment in pAS99. The transformants were crossed to a smcl-1 strain (5dAS98) and the resulting diploids were subjected to the tetrad analysis. In 10 tetrads analyzed, each contained two SMCI URA3 spores and two smcl-1 ura3 spores, indicating tight genetic linkage of the cloned sequence to the SMCI locus. The cloned DNA was hybridized to the pulsed field-gel blots of yeast chromosomes and to the lambda phage clones (from ATCC) with contiguous inserts of yeast genomic DNA. The results mapped the DNA fragment from pAS99 to the left arm of chromosome VI, tightly linked to CDC4, consistent with the previous genetic mapping of smcl-1 (Mortimer et al., 1989). Taken together these data demonstrated that the SMCI gene indeed was cloned.

SMCI Is an Essential Gene

Analysis of the pAS99 subclones established that the complementing activity was contained within a 4.2-kb Mlu1–NheI restriction fragment (Fig. 1). Convenient restriction sites were used to construct the smcl-Δ2 allele in which the SMCI gene was replaced with HIS3 (Fig. 1). This disruption was introduced in a diploid strain (AS153) by transformation selecting for His+. 40 meiotic tetrads from four independent transformants were dissected. All the tetrads segregated two viable and two inviable spores. All viable spores were His+, as expected if HIS3 marker disrupted an essential gene. The inviable spores did not have a defect in germination as they all germinated to form microcolonies of three to four cell bodies. When a strain (AS154) heterozygous for the disruption was first transformed with a plasmid pAS128 carrying SMCI and then subjected to meiotic analysis, the His+ segregants bearing plasmid marker LEU2 were observed. Mitotic stability of the pAS128 in these strains was 100% even under conditions nonselective for the plasmid marker. This plasmid could be replaced with other CEN plasmids only if they contained the SMCI gene. The smcl-Δ2

\[ \text{Figure 1. Subcloning and disruption of the SMCI gene. The top bar presents a schematic representation of the insert in pAS99 capable of complementing the smcl-1 mutation. Subclones were derived from pAS99 by making intraplasmid deletions of the insert or by moving pieces of the insert into YCpplac11 and pRS415. The subclones were tested for whether they could (+) or could not (−) complement the smcl-1 mutation. The arrow shows location of SMCI ORF. Only unique restriction sites or ones used for cloning are shown: A, Apal; B, BamHI; E, EagI; K, KpnI; M, MluI; N, NcoI; Nh, Nhel; S, SpeI; Sr, SacI; Sp, SphI; X, XbaI. CDC4 gene is located upstream of SMCI in the same orientation.} \]
deletion was shown by DNA sequencing to remove the entire SMCI coding region. Taken together, all of the above results indicated that SMCI function is essential for mitotic cell divisions. Another deletion (smcl-2) that encoded only the first 200 amino acids of SMCI protein was also a recessive lethal, indicating that the amino terminal part of Smclp was not capable of providing the essential SMCI function.

Loss of Artificial and Natural Chromosomes in smcl Mutants

Since the SMCI gene is essential for viability, it was possible to construct conditional lethal alleles. The plasmid pAS128 was mutagenized with hydroxylamine, and three temperature-sensitive smcl mutations (smcl-2, smcl-3, and smcl-4) were identified using the method of plasmid shuffling (see Materials and Methods). Because all mutations exhibited similar phenotypes (data not shown) and we could not guarantee their independence, further study was focused on just smcl-2. This allele was introduced into the genome to obtain stable ts strains (Materials and Methods). It was recessive to SMCI for growth at the nonpermissive temperature. Using the smcl-2 allele and the original smcl-1 allele, we re-investigated the role of the SMCI gene product in the transmission of both artificial and natural chromosomes.

First, we asked whether the loss of circular minichromosomes induced by smcl-1 was specific for circular minichromosomes with centromeres. Yeast have at least two sequences other than CEN DNA that promote an ordered segregation of circular plasmids during mitotic cell division: REP3 sequences from 2µ DNA (Murray and Szostak, 1983b) and telomere repeat sequences (TRS) (Longtine et al., 1992). Isogenic smcl-1 (pAS112/pAS136) and SMCI (pAS112/pAS135) strains were transformed with a set of plasmids marked with URA3 and bearing CEN, REP3, or TRS. The smcl-1 mutation had no effect on the mitotic stability of any plasmid other than the CEN plasmid (Fig. 2). This observation suggests that smcl-1 either specifically affects CEN-based segregation, or that the effect is detectable only when the plasmid is present in low copy number per cell, as in the case of the CEN plasmids.

Next we examined whether smcl-1 affected linear chromosomes. The previous study (Larionov et al., 1985) had shown that this mutation did not cause detectable loss of chromosome III. Similarly, we observed only marginal in-

crease in the rate of nondisjunction of chromosome VIII when we compared the smcl-1 strain with the completely isogenic SMCI strain (data not shown). However, this mutation did affect the mitotic stability of an artificial linear chromosome. Strains were constructed (laAS148, 5aAS194, and 5dAS194) that had identical genotype and contained the smcl-1 allele and the 90-kb chromosome fragment, CF110 (Palmer et al., 1990) were constructed. The mitotic stability of the chromosome fragment was determined by half-sector analysis (Koshland and Hieter, 1987). In these strains, the CF110 had a loss rate of 22 ± 2% per cell division, which is more than three times higher than the value obtained for congenic wild-type strains (6 ± 2%). Hence, smcl-1 was able to induce loss of short artificial linear chromosomes to a level similar to what we observe with circular minichromosomes.

Chromosome transmission in smcl-2 was also analyzed at 30°C. The mitotic stability of the circular minichromosome YCP41 in the smcl-2 strain was 85 ± 3% (94 ± 3% for isogenic SMCI strains), compared to only 62% in smcl-1. Thus, CEN plasmids were more stable in smcl-2 cells than in smcl-1 cells. When we made a homozygous smcl-2 diploid, we observed that this diploid could mate with haploid cells of either mating type at a much greater frequency than homozygous SMCI or smcl-1 diploids. This phenotype indicated that information at the MAT locus was being lost in the smcl-2 diploids. To investigate further the loss of chromosomal information in smcl-2 cells, we made congenic SMCI (AS203) and smcl-2 (AS241) diploids that were heterozygous at the loci on the left (LEU2/leu2) and right (MATa/MATα) arms of chromosome III. We used these strains to measure the rate of chromosome III loss and the rate of recombination in the LEU2–CEN3 interval (see Materials and Methods). We did not detect any recombination between the CEN3 and LEU2 in diploid smcl-2 (<10⁻²) or in SMCI (<6 × 10⁻³) cells. In contrast, chromosome III loss in smcl-2 cells (2 × 10⁻⁵) was happening at least 50 times the rate in SMCI. Therefore, the smcl-2 mutation differed from the smcl-1 mutation in two respects; it had little effect on the loss rate of circular minichromosomes but dramatically increased the loss rate of chromosome III. Taken together these studies of smcl mutants indicate that the Smcl protein is required for transmission of both natural and artificial chromosomes.

Requirement of SMCI Function at a Discrete Stage of the Cell Cycle

We used the smcl-2 mutants to examine a consequence of the Smclp inactivation on cell viability and cell morphology. After 2–3 h exposure to the nonpermissive temperature (36°C), both haploid and diploid smcl-2 cells stop dividing (Fig. 3). The viability of mutant cells decreased significantly with time spent under nonpermissive conditions such that after 4 h at the nonpermissive temperature, only 3% of the cells were viable (Fig. 4). No significant differences between the kinetics and absolute level of the lethal effect were observed in the haploid and diploid smcl-2 strains. As the doubling time of yeast cells at 36°C is ~1.5–2 h, the lethal event in most of the smcl-2 cells apparently occurred during either the first or the second cell division at the nonpermissive temperature.
The parameters of cell shape, DNA distribution, and spindle structure in *smc1-2* and *SMC1* cells were monitored microscopically (Figs. 5 and 6). After 3 h at the nonpermissive temperature (36°C) neither haploid or diploid *smc1-2* cells were uniform for any of these parameters, indicating that *smc1-2* mutation does not confer a uniform cdc-like phenotype. However, there were two distinct differences between the appearance of *smc1-2* and *SMC1* cells at 36°C. In *SMC1* cultures about 30% of the cells were large budded; the vast majority of these had two segregated DNA masses at the end of elongated spindles. This cell type was dramatically reduced in *smc1-2* cultures. Instead, the majority of large budded cells had a single DNA mass at the bud neck. The DNA mass was either undivided or pinched in appearance and the corresponding spindles were short or predominantly intermediate in length, with some of them broken in appearance (Fig. 5, B and C). FACS analysis at 36°C showed that a culture of *smc1-2* diploids had a greater fraction of cells with a G2 DNA content than was observed in the *SMC1* culture (data not shown). These observations suggest that at the nonpermissive temperature *smc1-2* cells are defective in a nuclear division step. Consistent with this, germination of the *smc1-2* (deletion) spores gave rise to 3-4-cell bodies with a single nucleus (data not shown). No obvious defects in morphology or number of cellular organelles in *smc1-2* were detected by electron microscopy.

The fact that *smc1-2* cells undergo rapid cell death allowed us to address whether the Smclp performs an essential function at one or more stages of the cell cycle. To do this we asked whether *smc1-2* cells remained viable after a transient inactivation of Smclp at different points of the cell cycle (see legend to Fig. 7). Several congenic *smc1-2* haploids were arrested in G0 (starvation), G1 (alpha factor), S (hydroxyurea), or G2-M (nocodazole). While arrested, these strains were exposed to the nonpermissive temperature in order to deplete Smclp activity. Cells were released from an arrest at the permissive temperature, and the percent of viable cells was determined. When Smclp activity was depleted in G2-M, >80% of *smc1-2* cells died (Fig. 7). In contrast, when Smclp activity was depleted in G0, G1, or in S, anywhere between four- to sevenfold fewer cells died; the residual cell death in some cultures could be explained by inability to achieve complete cell cycle arrest with these reagents (Fig. 7). These results suggest that *SMC1* function is essential for cell viability during M but not G0, G1, or S phases of the cell cycle.

DNA lesions due to damage or incomplete replication induce a cell cycle checkpoint that transiently arrests cells with a large bud, undivided nucleus, and a G2 content of DNA. This arrest apparently requires the *RAD9* protein, as it does not occur in *rad9* mutants (Weinert and Hartwell, 1988). To address whether any of the *smc1-2* cell types observed at the

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*Figure 3.* Growth of *smc1-2* cultures under permissive and restrictive temperatures. Logarithmic cultures of a *smc1-2* haploid (laAS172) and homozygous *smc1-2* diploid (AS175) strains were grown at 23°C and then a portion of these cultures were shifted to 36°C. Aliquots were removed immediately after the shift (0 h) and at 2-h intervals. The cell number in the cultures was determined with a hemocytometer. Error bars indicate the standard deviation in cell number measurements.

*Figure 4.* Temperature dependent lethality of *smc1-2*. Logarithmic cultures of *smc1-2* haploid (laAS172) and diploid (AS175) strains were shifted from 23 to 36°C. Aliquots were removed immediately after the temperature shift and at 1-h intervals and then subjected to mild sonication. For each aliquot, the total number of cells was determined and cell viability was estimated by colony formation on plates. The percentage of viable cells (viable cells/total cell number) was plotted as function of time after temperature shift.

*Figure 5.* Micrograph of *smc1-2* cells and *SMC1* cells. (A) Two parallel logarithmic cultures (YEPD medium) of *smc1-2* diploid strain were set up. One culture was shifted from 23 to 36°C. Aliquots of both cultures were fixed in 3 h after the shift. Fixed cells were stained with DAPI to visualize DNA (DAPI) and also were examined with Nomarski optics (Nom.). *SMC1* cells grown at 23°C were similar to *SMC1* cells grown at 36°C and to *smc1-2* cells grown at 23°C (data not shown). (B) Fixed cells from *SMC1* (AS153) and *smc1-2* (AS195) diploid cultures (36°C, 3 h) were stained to visualize DNA (DAPI) and microtubules (Tubulin). Arrows point at the cells with elongated spindles and segregated nuclear masses (anaphase) in *SMC1* culture and at the cells with cut nuclei and corresponding short or broken spindles in *smc1-2* cells. (C) Enlargements of arrested (36°C, 3 h) *smc1-2* diploid cells (AS195) showing a nuclear DNA and spindle aberrations, presumably representing different stages of the abortive cytokinesis (see Discussion). Bar, 10 μm.
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nonpermissive temperature appeared as a consequence of DNA damage, we first asked whether cell type distribution in smcl-2 cells was altered in a rad9 background. In fact, cell type distribution in smcl-2 strains and smcl-2 rad9 strains were indistinguishable; in particular, the fraction of cells with a large bud and single nucleus did not change. Therefore, the accumulation of G2-M cells in smcl-2 was not dependent upon the RAD9 checkpoint.

In addition, we monitored the state of nuclear DNA molecules isolated from SMC1 and smcl-2 at 36°C. Different forms of circular plasmids (nicked, closed, and topologically intertwined) can be separated by gel electrophoresis. When plasmid DNA was recovered from smcl-2 and SMC1 cells, the distribution of plasmid DNA molecules among the different forms was indistinguishable (data not shown). This result contrasts with ligase and topoisomerase-II mutants where dramatic changes in distribution of plasmid forms are evident (Koshland and Hartwell, 1987). Migration of chromosomal DNA in a pulse-field gel is a very sensitive measure of DNA structure as aberrant DNA forms such as replication intermediates fail to enter the gel (Hennessy et al., 1991). Chromosomal DNA from smcl-2 mutants exhibited normal mobility in a pulse-field gel (data not shown). Therefore, by these genetic and molecular assays, the defect in nuclear division in smcl-2 cells was not apparently a consequence of DNA damage or incomplete DNA replication.

Sequence Analysis of the SMC1 Gene
To learn more about possible function of SMC1 gene product, the primary DNA sequence of the gene was determined. A single open reading frame was identified, capable of coding for a 1225-amino acid residue protein with predicted molecular mass of 141 kD (Fig. 8). The sequence of the Smclp was analyzed using GCG package (version 7; Genetics Computer Group, Inc., Madison, WI). Residues 33–39 (GPNGSGK) and 79–83 (DNEG) matched two of the consensus sequences found in most NTP-binding proteins (Diver et al., 1987). No match was found to a third element con-
Figure 8. Sequence analysis of SMCI gene. Primary DNA sequence of SMCI gene and putative translation product are shown, starting 150 bp downstream of the first Spel site (Fig. 1). Matches to the NTP-binding site consensus (GFGNSGK) and the nuclear localization signal (KKKKR) are shown in italics and underlined. Unique recognition sites for MluI, BamHI, XbaI, EagI, and NheI are shown. The putative sites for posttranslational modification (Bairoch, 1992) were also identified. The position of each putative site is indicated by an asterisk. 

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phenylalanine by site-directed mutagenesis. The resulting mutant gene was able to complement minichromosome loss in smcl-1, temperature sensitivity of smcl-2, and inviability of smcl-Δ2. These results demonstrate that Tyr^{300} is not essential for Smclp function and that similarity between region 287-301 and the topoisomerase consensus may be fortuitous.

**SMCI Is an Evolutionary Conserved Gene**

We carried out comparison of the Smclp amino acid sequence to amino acid sequences of other proteins with BLASTP and TBLASTN programs (Altschul et al., 1990). Related sequences were found not only in yeast, but in mycoplasma, purple nonsulfur bacteria, and mouse as well (Fig. 10). The sequence from *Mycoplasma hyorhinis* was a full-length gene encoding a 115-kD protein of unknown function (Notarnicola et al., 1991). This protein shared with Smclp a common type of organization: two putative central coiled-coiled regions flanked by putative globular regions at the amino and carboxy termini (Fig. 9). Further indication of this common organization was that a putative NTP-binding pocket was located at the same position in these two proteins, and a high level of identity was found around the NTP-binding region and throughout the COOH-terminal domain (Fig. 10). The major difference between the two proteins was that the Smclp is longer by 250 residues. These additional residues appear to be equally distributed in the two coiled-coil regions. The similar structural organization and the amino acid identity in the globular domains indicates that the Smclp and Mycl15p have a common evolutionary origin.

In addition, a 57-residue incomplete ORF from mouse (Varnum et al., 1991) showed extensive identity with the putative NTP-binding regions of Smclp and Mycl15p. The degree of similarity of these sequences to each other is significantly greater than the similarity to NTP-binding regions of other proteins suggesting that a mammalian homologue of Smclp may exist.

The COOH-terminal regions of Smclp and Mycl15p showed significant similarity to polypeptides that were conceptual products of incomplete open reading frames from budding yeast and purple bacteria (Fig. 10). The high level of identity between the Smclp peptide (residues 1129-1165) and the corresponding regions from the other three polypeptides suggests that these proteins could be considered an evolutionary related group, and the peptide can be used as a signature for this family. We named this peptide a “DA-box” after the highly conserved aspartate (D) and alanine (A) residues present in it (Fig. 10). The analysis of the COOH-terminal region of Smclp by several algorithms for predicting secondary structure (Chou and Fasman, 1978; Garnier et al., 1978; Rost and Sander, 1993) suggested that it contained a putative helix-loop-helix structure. The first helix and loop (Fig. 10) was comprised of the residues in the DA-box. The second helix was comprised of a dozen residues immediately following the DA-box. This helix-loop-helix structure was common to all of the homologues analyzed.

![Figure 9](image-url)

**Figure 9.** Prediction for coiled-coil structure for Smclp and Mycl15p. Probability (%) of coiled-coil formation in Smclp and Mycl15p was generated by NewCoil program (Lupas et al., 1991) using the maximal window width (24 residues).

![Figure 10](image-url)

**Figure 10.** Alignment of amino- and carboxy-termini of Smclp to potentially homologous polypeptides. Alignment of the most conservative parts of Smclp is shown to: *M. h. 115p*, *Mycoplasma hyorhinis* 115 kD protein (Notarnicola et al., 1991); mouse URF; partial ORF (-1 frame) from mouse cDNA clone (Varnum et al., 1991); Rh. r. URF, *Rhodospirillum rubrum* partial ORF (+3 frame) located upstream of F(0) gene cluster (Falk and Walker, 1988); S. c. ARS, yeast partial ORF (-1 frame) from ARS-containing clone of chromosome VI (Shirahige et al., 1993). Blocks of alignment are presented as appeared in BLAST report, with consensus sequences generated from pairwise comparison of Smclp to putative homologues. Percent identity is shown only for the sequences displayed. Boundaries between the helices and the loop are shown according to prediction (Rost and Sander, 1993) made for Smclp. Region designated “DA-box” is marked with arrows.
Antibodies Specific for Smclp Recognize a 165-kD Protein

To characterize the \textit{SMCI} gene product, polyclonal rabbit antibodies were raised against the \textit{E. coli}-produced PEPl85 (residues 794–1225 of Smclp). In addition, we introduced the myc–epitope tag (Roth and Gall, 1989) in frame into one of the putative coiled-coil regions of the Smclp (predicted molecular mass 151 kD). The \textit{SMCI::myc} gene on the CEN plasmid pAS211 was able to complement \textit{smcl-A2} and \textit{smcl-2} alleles, indicating that the fusion protein was functional in vivo. Affinity-purified rabbit anti-Smclp antibodies as well as monoclonal anti-myc antibodies recognized bands of 165 and 178 kD, respectively, in total cell extracts (Fig. 11). The apparent molecular weight of the proteins in these bands were close to the predicted molecular weights of Smclp and Smcl-mycp, respectively. \textit{SMCI} and \textit{SMCI::myc} were put under \textit{GAL/0,~\textGamma{}-controlled} promoter and integrated into the chromosome of \textit{SMCI} diploid AS153. These cells grew at normal rates in the presence of galactose (data not shown). When extracts were prepared from these induced cells, intensity of the 165- and 178-kD bands increased (Fig. 11). These data strongly suggest that the 165- and 178-kD proteins recognized by the antibodies were indeed the product of the \textit{SMCI} and \textit{SMCI::myc} genes, respectively, and that overproduction of Smclp apparently was not toxic to cells. Finally, using these antibodies we analyzed the amount of Smclp in protein extracts made from a panel of cdc-mutants arrested at specific points of the cell cycle. No differences were observed (data not shown) suggesting that the amount of Smclp does not vary during the cell cycle.

We have used these antibodies in an attempt to localize the Smclp in the cells by indirect immunofluorescence (Fig. 12 \textit{A}). When Smclp was expressed from its own promoter we observed weak nuclear staining and some diffuse cytoplasmic staining in most cells. Some small bright dots that usually colocalized with the nuclear DNA were also observed. As the \textit{SMCI} gene is essential, we were unable to stain the cells with the gene deleted to address whether the observed staining pattern was specific for Smclp. As an alternative, we constructed strains that were deleted for the chromosomal \textit{SMCI} gene and contained either the \textit{SMCI} gene or the \textit{SMCI::myc} gene on a centromere-based plasmid. These strains were subjected to indirect immunofluorescence using anti-myc antibodies. In cells expressing the Smcl-mycp, the anti-myc antibodies gave nuclear and cytoplasmic staining that was very similar to the pattern observed for cells stained with the polyclonal anti-Smclp antibodies (Fig. 12 \textit{B}). In the same cells that did not contain the Smcl-myc protein, the anti-myc antibodies failed to give any staining (Fig. 12 \textit{C}), demonstrating that the staining observed with the anti-myc antibodies was specific for the Smcl-mycp. We did not observe the bright nuclear dots when the cells expressing Smcl-mycp were stained with the anti-myc antibodies (Fig. 12 \textit{B}). Therefore, relevance of the dots for Smclp is unclear. Taking
all the immunolocalization results together, we suggest that the Smcl protein is concentrated in the nucleus but also present in the cytoplasm.

Discussion

Smcl Protein Has an Essential Activity Required for Chromosome Segregation

Here we demonstrate that the SMCI gene encodes a 141-kD protein whose function is essential for mitotic cell divisions in S. cerevisiae. We show that a new allele, smcl-2, causes improper transmission of natural chromosomes while the smcl-1 mutation interferes with accurate transmission of circular minichromosomes and linear chromosome fragments (this study; Larionov et al., 1985). Hence, Smclp is required for the mitotic transmission of artificial and natural chromosomes.

The original observation that smcl-1 mutants undergo frequent minichromosome nondisjunction (Larionov and Strunnikov, 1987) indicated that Smclp might be required for chromosome segregation. Four additional observations in this study support this conclusion. First, previous analysis of a large number of yeast mutants has indicated that defects in replication factors such as DNA-polymerase and DNA-ligase increase both chromosome recombination and loss (Hartwell and Smith, 1985; Palmer et al., 1990). In contrast, defects in segregation components, such as the major protein of mitotic spindle, tubulin, dramatically elevate chromosome loss but not recombination (Huffaker et al., 1988). As smcl mutants exhibited increased rates of chromosome loss but no detectable increase in mitotic recombination (this study), they behave most like other mutants defective in chromosome segregation. Second, when yeast cells are depleted for SMCI function, almost half of the cells in the population acquire a cell morphology and DNA content indicative of a delay or an arrest in the G2-M portion of the cell cycle. This delay occurs in the absence of RAD9 function. RAD9-independent G2-M delays are characteristic of mutants defective in segregation but not in DNA replication or repair components (Weinert and Hartwell, 1988). Third, smcl-1 induces loss of minichromosomes that use centromere-based segregation but not those that use 2μ plasmid or telomere-mediated segregation. This specificity is also consistent with Smclp functioning in some aspect of centromere-based segregation. Fourth, temporary loss of SMCI activity in asynchronously dividing cells leads to their death. More importantly, cell death is also observed when SMCI function is transiently inactivated in cells arrested in G2-M. However, far fewer cells die when SMCI activity is transiently disrupted in cells synchronized in G0, G1, or S. These results suggest that the essential function of Smclp occurs at the time of mitosis. While individually each of these observations has alternative interpretations, taken together they strongly suggest that Smclp has an essential function in yeast nuclear division and/or chromosome segregation.

Mutations that perturb segregation components often cause cells to arrest uniformly in the cell cycle with a 2C content of DNA, a large bud, an undivided nucleus, and a short spindle. While a significant fraction of smcl-2 cells acquire this classical G2-M arrest, many cells appear to have a partially elongated or broken spindle with the nucleus pinched into two masses connected by a thin thread of DNA. In addition, a significant fraction of unbudded and small budded Gl-like cells are also observed. The failure to observe a uniform arrest in smcl-2 mutants has several explanations. It is possible that residual SMCI function persists at the nonpermissive temperature; eventually the cell accumulates enough of this residual function to proceed through an anaphase. However, this model does not explain why, at the nonpermissive temperature, smcl-2 cells die rapidly and fail to exhibit the telophase morphology expected for the successful completion of a normal anaphase (two DNA masses at the end of a spindle that traverses the length of the cell). As an alternative model, smcl-2 cells may never complete anaphase before they undergo cytokinesis. The cells with the pinched nuclei would be in the middle of such precocious cytokinesis while the Gl-like cells would be the lethal products of such abortive cytokinesis. A similar cut phenotype was reported in segregation mutants of Schizosaccharomyces pombe mutants (Samejima et al., 1993), in topoisomerase II (Holm et al., 1989), and a centromere protein mutant (Doheny et al., 1993) in budding yeast.

Smclp Represents a New Family of Proteins

Comparison of the predicted amino acid sequence of Smclp with other proteins in databases indicates that this protein, as a whole, is not a homologue of any protein with known biochemical function. However, analysis of primary and secondary structure within the predicted Smclp amino acid sequence, suggests that Smclp is a member of a new family of structurally similar proteins. Based upon the comparison of two members of the family (Smclp and Mycl115p), a typical member of the family apparently consists of two large central domains capable of forming coiled coil. This central region is flanked by NH2-terminal NTP-binding region and a COOH-terminal domain containing a novel, highly conserved 35-residue peptide, or DA-box. From this comparison we conclude that members of this family exist at least in both prokaryotes and lower eukaryotes. While no other full-length homologue was found in databases, the putative NH2- and COOH-terminal globular regions of SMCI were highly similar to polypeptides that were the putative products of incomplete open reading frames from mouse, budding yeast, and purple bacteria. Furthermore, another full-length homologue has been discovered recently in C. elegans (P. Chuang and B. Meyer, personal communication). Therefore, it seems likely that the members of the DA-box family are ubiquitous.

Potential Functions of SMCI

The primary molecular function of the Smclp remains obscure. However, certain models for its function could be suggested based upon its proposed structure. The juxtaposition of a putative NTP-binding domain with an extensive coiled-coil region in Smclp is highly reminiscent of NTP-dependent motor proteins kinesin and myosin. Indeed, putative protein motors have been implicated in chromosome segregation (Hagan and Yanagida, 1990; Saunders and Hoyt, 1992; Yen et al., 1992). However, Smclp shares no homology with any known mechanochemical domain. Therefore, if Smclp is a force generating protein, it represents a new class. Interestingly, the RADS0 product of yeast (Alani et al., 1989) and
the E. coli protein mukB (Niki et al., 1992) share a similar organization with these motors and Smclp. While no motor activity has been identified for Rad50 and mukB proteins, these proteins are known to be important in yeast meiosis and in E. coli nucleoid segregation, respectively.

Alternatively, models for Smclp function could be derived from the phenotype of smcl mutants and the high degree of sequence conservation between Smclp and several proteins from evolutionary distant organisms. As SMCI function is required for proper segregation of nuclear DNA in yeast, and Smclp has a bacterial homologue, Smclp may perform a segregation function that is common to both prokaryotes and eukaryotes. Clearly, a substrate common to chromosome segregation in all cellular organisms is a chromosome itself. Thus, Smclp-like proteins would be involved in some ubiquitous process facilitating chromosome segregation, such as restructuring a nucleoskeleton (cytoskeleton in bacteria), assembly of partition loci (centromeres in yeast), organization of chromosome structure (condensation or decondensation), or association of sister chromatids. The idea that Smclp acts at the chromosomal level is supported by its nuclear localization and the general structural similarity of Smclp to Rad50p, a molecule known to be involved in chromosome metabolism in meiotic cells (Alani et al., 1990). Moreover, we have recently identified a high copy suppressor of the temperature-related lethality of smcl-2. DNA sequencing of the suppressor gene revealed an open reading frame with a HMG1 motif common for nonhistone chromosomal proteins (Kolodrubetz, 1990). While these observations and the conserved structure of DNA would make chromosomes a likely target for Smclp, we can not rule out the possibility that components of the segregation machinery other than chromosomal DNA are actually conserved between eukaryotes and prokaryotes. We anticipate that validity of the hypothetical models presented will be resolved by ongoing biochemical analysis of Smclp function in yeast, as well as by studies on potential Smclp homologues in other organisms. Regardless of the particular function of Smclp, the prospect of a novel, yet highly conserved function in cell division cycle is very interesting.

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