Regulation and Intracellular Localization of *Saccharomyces cerevisiae* Strand Exchange Protein 1 (Sep1/Xrn1/Kem1), a Multifunctional Exonuclease

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The *Saccharomyces cerevisiae* strand exchange protein 1 (Sep1; also referred to as Xrn1, Kem1, Rar5, or Stpβ) catalyzes the formation of hybrid DNA from model substrates in vitro. The protein is also a 5′-to-3′ exonuclease active on DNA and RNA. Multiple roles for the in vivo function of Sep1, ranging from DNA recombination and cytoskeleton to RNA turnover, have been proposed. We show that Sep1 is an abundant protein in vegetative *S. cerevisiae* cells, present at about 80,000 molecules per diploid cell. Protein levels were not changed during the cell cycle or in response to DNA-damaging agents but increased twofold during meiosis. Cell fractionation and indirect immunofluorescence studies indicated that >90% of Sep1 was cytoplasmic in vegetative cells, and indirect immunofluorescence indicated a cytoplasmic localization in meiotic cells as well. The localization supports the proposal that Sep1 has a role in cytoplasmic RNA metabolism. Anti-Sep1 monoclonal antibodies detected cross-reacting antigens in the fission yeast *Schizosaccharomyces pombe*, in *Drosophila melanogaster* embryos, in *Xenopus laevis*, and in a mouse pre-B-cell line.

Sep1 was identified in a biochemical search for *Saccharomyces cerevisiae* DNA recombination proteins (39) and identified in a similar screen as Stpβ (Dst2) (10). It has also been identified as exoribonuclease I (Xrn1), which is thought to be involved in RNA turnover (22, 47, 55). The *SEP1* gene has also been identified as *KEM1*, a gene that affects nuclear fusion during mating (35), and as *RAR5*, a gene involved in the stability of replication-defective plasmids (37; reviewed in reference 32).

Sep1 displays homologous pairing and strand exchange activities in vitro (8, 10, 20, 39; reviewed in references 19 and 41) and contains an intrinsic 5′-to-3′ exonuclease (25). These properties have suggested a role in DNA recombination. Although *sep1* mutants do not show significant defects in mitotic recombination (3, 11, 35, 62) or in mating-type switching (57), the meiotic recombination defects observed in *sep1* mutants have been interpreted to indicate that Sep1 plays a direct role in meiotic DNA recombination (3, 11, 62, 63). Kem1 (Sep1) has recently been described as a nuclease specific for G4 tetrastranded DNA (45). It was proposed that the meiotic arrest observed in *sep1* mutants (3, 63) arises from an inability to initiate pairing of homologous chromosomes via the formation of interchromosomal G4 tetrastranded DNA (45). In addition, Sep1 could play a role in the function of telomeres which may contain G4 tetrastranded DNA. However, the correlation of in vitro G4 tetrastranded DNA binding with in vivo function remains to be demonstrated. Genetic arguments have suggested that Sep1 carries out other essential meiotic functions in addition to recombination (3, 63). Consequently, it is possible that the meiotic arrest is regulatory in nature and that the resulting recombination defects are a consequence of the arrest point (3, 62, 63).

Genetically, *SEP1* was identified as *KEM1* in a screen for mutants enhancing the nuclear fusion defect of *kar1* mutants (36). In addition, *kem1* mutants were shown to have an elevated rate of chromosome loss, to be hypersensitive to benomyl, a microtubule-destabilizing drug, and to be sensitive to nitrogen starvation. Impaired microtubule function was proposed as the primary defect leading to aberrant nuclear and cytoplasmic functions (36). *SEP1* was also identified as *RAR5* in a screen for mutations allowing *CEN ARS* plasmids with defective *ARS* sequences to be stably maintained in the cell (38). In the absence of the RNase of Sep1, RNA molecules that remain annealed to the plasmid DNA may serve as replication primers, possibly alleviating the *ARS* defect (38). Alternatively, the slower growth of *sep1* mutants could suppress the *ARS* defect of the mutant plasmids.

Sep1 was also purified and identified as Xrn1, a 5′-to-3′ exonuclease thought to have a role in RNA turnover in *S. cerevisiae* (43, 55). Recent studies of transcript turnover have shown that different transcripts accumulate as unadenylated, uncapped species in *xrn1* mutants at two- to eightfold the levels observed in wild-type cells (22). These results are consistent with the finding that *xrn1* mutants display reduced rates of 5′-to-3′ exonuclease degradation of the *MFα1* transcript following deadenylation and decapping (47) and suggest that Xrn1 is at least in part the nuclease responsible for this degradation. In addition, *xrn1* mutants accumulate products of 20S rRNA processing, suggesting that the protein has a broad RNA substrate specificity (17, 56).

The multiple apparent roles of Sep1 are difficult to reconcile with a simple model of Sep1 function. Sep1 may be a multifunctional protein involved in nuclear and cytoplasmic DNA and RNA metabolism, and/or some phenotypes of *sep1* mutants may be the indirect consequence of some other primary lesion(s) in these mutants. The various suggested roles for Sep1 lead to predictions about Sep1, especially with respect to cellular localization. In this study, we have examined the reg-

TABLE 1. *S. cerevisiae* strains used in this study

Strain[a]	Genotype	Reference or source
BJ5464	**a** *ura3-52 trp1 leu2Δ1 his3Δ200 pep4::HIS3 prb1Δ1.6R can1 GAL*	27
L42	**a**/**a** *his4/his4 ura3-52/ura3-52 lys2-173R2/lys2-173R2 trp1/TRP1*	F. Winston, Harvard Medical School
LDY1	**a**/**a**/α/α *lys1-1/LYS1/LYS1/LYS1 his4D5/his4D29/HIS4/HIS4 arg4/ARG4/ ARG4/ARG4 cry1/CRY1/CRY1/CRY1*	L. Davis, Massachusetts Institute of Technology
NKY211	**a** *ho::LYS lys2*	N. Kleckner, Harvard University
NKY921	**a** *ho::LYS2 lys2 ura3Δ::hisG trp1::hisG leu2::hisG ade2::TnLK*	N. Kleckner, Harvard University
RKY1154	**a**/α *ho::hisG/ho::hisG lys2/lys2 ura3/ura3 leu2::hisG/leu2::hisG*	
RKY1303	**a**/α *ho::hisG/ho::LYS2 lys2/lys2 ura3/ura3 leu2::hisG/LEU2 sep1::Tn10LUK79-2/SEP1*	62
RKY1308	**a**/α *ho::LYS2/ho::LYS2 lys2/lys2 ura3/ura3 leu2::hisG/leu2::hisG his4-X/his4-B*	
RKY1957	**a**/α *ho::LYS2/ho::LYS2 lys2/lys2 ura3/ura3 leu2::hisG/leu2::hisG his4-X/his4-B sep1Δ-URA3/sep1Δ-URA3*	
WDHY150	**a**/α *ho::LYS2/ho::LYS2 lys2/lys2 ura3/ura3 leu2::hisG/leu2::hisG his4-X/his4-B*	3
WDHY152	**a**/α *ho::LYS2/ho::LYS2 lys2/lys2 ura3/ura3 leu2::hisG/leu2::hisG his4-X/his4-B sep1Δ-LEU2/sep1Δ-URA3*	3
WDHY167 and WDHY193 (2 independent isolates)	α/α *ho::LYS2/ho::LYS2 lys2/lys2 ura3/ura3 leu2::hisG/leu2::hisG his4-X/his4-B*	

[a] All strains except L42, LDY1, and BJ5464 are derivatives of SK-1 from strains originally kindly supplied by N. Kleckner.

ulation, abundance, and localization of Sep1 to gain some insight to its function in *S. cerevisiae*. Surprisingly, we find that Sep1 is constitutively expressed at relatively high levels and is found primarily in the cytoplasm.

MATERIALS AND METHODS

Strains, media, and genetic and physiological methods. The *S. cerevisiae* strains used are listed in Table 1. *E. coli* RK1400 (59) was the host for plasmids. Rich (YPD), presporulation (YPAc), minimal (SD), synthetic complete, YPG, YPL, and sporulation media were prepared as described previously (3, 50). The α/α diploid control strains WDHY167 and WDHY193 (Table 1) were obtained as nonmating derivatives of WDHY150 by mitotic recombination. Chromosome loss was excluded by demonstrating the presence of the *his4* heteroalleles (*his4* and *MAT* are both located on chromosome III). The α/α strains showed no sporulation under standard conditions and the expected segregation and meiotic lethality in triploid crosses with NKY921. Mating, sporulation, and tetrad analysis were performed as described previously (50). Meiotic time course analysis has been described elsewhere (3, 48, 62). α-Factor arrest-release experiments were performed as described previously (12), using strain L42 (Table 1). Aliquots were taken for fluorescence-activated cell sorting (FACS) analysis, for microscopic examination, for protein extracts, and for RNA preparation. RNA was analyzed as described previously (51), using specific probes (*SEP1*, 3.1-kb *Sac*I-*Cla*I fragment [62]; *TRT1*, 2.4-kb *Sac*I fragment containing *HTA1* and *PRT1* [51]; *RPA1*, *Bam*HI-*Mlu*I fragment of pRPA1 [21]). Sep1 levels after UV (0 to 600 J/m², 0 to 6 h) or methyl methanesulfonate (0.01%, 0 to 300 min) treatment of cells (12) were monitored in strains RKY1154 and BJ5464 (Table 1).

Enzymes. p132^{SEP1} (indicating the M_r-132,000 proteolyzed form; reviewed in reference 19) was purified through fraction V as described previously (39). For the second screen of the hybridoma lines, we further purified p132^{SEP1} fraction V by chromatography on double-stranded DNA–cellulose to obtain fraction VI with ≥95% purity as judged from Coomassie blue-stained gels. p175^{SEP1} (indicating the M_r-175,000 full-length form and referred to in this report generally as Sep1; reviewed in reference 19) was purified through fraction V as described previously (25).

Monoclonal and polyclonal antibodies. RBF DNJ female mice were immunized three times with 0.5 mg of p132^{SEP1} fraction V, and splenocytes were fused to a myeloma cell line derived from FOX/NY (61). Fusion products were plated and tested by two consecutive rounds of indirect enzyme-linked immunosorbent assay using first p132^{SEP1} fraction V and then fraction VI as antigens. Clones were isolated by two rounds of limited dilution, resulting in 18 stable hybridoma lines. The immunizations, fusions, initial screening, and cloning were done by the Harvard University Monoclonal Antibody Facility. Individual anti-Sep1 monoclonal antibodies (MAbs) were purified from the supernatant of hybridoma cultures by protein G-Sepharose affinity chromatography. The isotype of each MAb was determined with mouse typer kit (Bio-Rad). Anti-ribosomal protein L3 MAb 7.1 was from J. Warner; MAb 2.3b, specific for Nsr1 (6), was a gift from C. Copeland and M. Snyder. A rabbit anti-Sep1 polyclonal antiserum was prepared by Pocono Rabbit Farm and Laboratory (Canadensis, Pa.), using Sep1 (fraction V, ≥95% pure [25]). Two rabbits were each injected intradermally with 200 μg of Sep1 with Freund's complete adjuvant on day 1. Three subsequent injections with 100, 50, and 25 μg were done with incomplete adjuvant. Serum from one

rabbit was then affinity purified against Sep1 fraction V. Partially purified polyclonal anti-*S. cerevisiae* topoisomerase II (14) and purified *S. cerevisiae* topoisomerase II were gifts of J. Lindsley and J. Wang. Rhodamine-conjugated goat anti-rabbit and Cy3-conjugated donkey anti-mouse antibodies were from Jackson ImmunoResearch Laboratories, Inc.

Immunological methods. For Western blot (immunoblot) analysis, proteins were separated on sodium dodecyl sulfate (SDS)-polyacrylamide gels and transferred to nitrocellulose (0.2-μm pore size; Schleicher & Schuell) or Immobilon-P (Millipore) membranes. Antigens were detected by using the ProtoBlot system (Promega). Quantitation of Western blots was performed by scanning the filter with a Hewlett-Packard Scanjet controlled by an Apple Macintosh IIci. For immunofluorescence, log-phase *S. cerevisiae* cultures in YPD or synchronous meiotic cultures in sporulation medium were fixed by the addition of formaldehyde solution to 3.7% and incubation for 40 min. The fixed cells were then washed twice with KSorb (1.1 M sorbitol, 0.1 M KPO₄ [pH 6.6]) and spheroplasted in KSorb plus 10 mM dithiothreitol and 0.1 mg of Zymolyase T100 (Seikagaku Kogyo Co. Ltd.) per ml for ≤20 min at 30°C. The spheroplasted cells were washed twice with KSorb plus 1 mM phenylmethylsulfonyl fluoride and put onto polylysine-coated slides. Samples were permeabilized by placing the slides in ice-cold methanol for 6 min and ice-cold acetone for 30 s and allowed to air dry. The samples were blocked in phosphate-buffered saline (PBS) plus 5% bovine serum albumin (BSA) overnight at 4°C. After one wash with PBS plus 0.1% BSA, the primary antibody, diluted appropriately in PBS plus 0.1% BSA, was applied for 2 to 4 h at room temperature. The cells were washed three times with PBS plus 0.1% BSA, and then the secondary antibody (diluted 1:500 for rhodamine-conjugated goat anti-rabbit and 1:300 for Cy3-conjugated donkey anti-mouse in PBS plus 0.1% BSA) was applied for 1 to 2 h. The cells were washed twice with PBS plus 0.1% BSA. DNA was stained by applying 1 μg of 4′,6-diamidino-2-phenylindole (DAPI) per ml in PBS plus 0.1% BSA for 1 min and then washed three times in PBS plus 0.1% BSA. The cells were mounted in Aqua-Poly Mount (Polysciences, Inc.), and indirect immunofluorescence was photographed on a Zeiss Axioskop microscope using a 100× objective with Kodak T-max 400 film.

Miscellaneous methods. Protein concentrations were determined by the method of Bradford (4). SDS-polyacrylamide gel electrophoresis (PAGE), done as described previously (42) with 0.75-mm-thick gels, was followed by staining with Coomassie brilliant blue R-250. Preparation of small-scale extracts of *S. cerevisiae* cells and flow cytometry (FACS) analysis of *S. cerevisiae* cells were done essentially as described previously (23, 25). Cell fractionation was done by the method of Aris and Blobel (2), using strain RKY1308 (Table 1).

RESULTS

Characterization of the MAbs. The specificity of each of the anti-Sep1 MAbs was demonstrated in Western blotting experiments (Fig. 1). A mix of all of the MAbs recognized both intact Sep1, p175^{SEP1}, and the proteolyzed form, p132^{SEP1} (see Materials and Methods) with high sensitivity (Fig. 1, lanes 1 through 4). The MAb mix was specific for Sep1: a single band which comigrated exactly with the purified p175^{SEP1} was detected from extracts of vegetative wild-type cells (lane 6), and
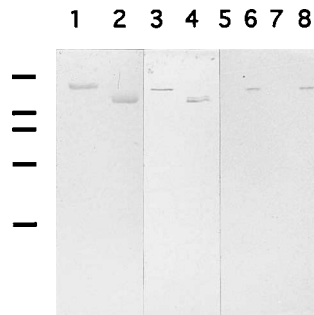
FIG. 1. Electrophoretic analysis of Sep1. Lanes: 1 and 2, Coomassie blue-stained SDS-polyacrylamide gel with 1 μg of p175^{SEP1} (lane 1) and 1 μg p132^{SEP1} (lane 2); 3 and 4, Western blot analysis of 50 ng of p175^{SEP1} (lane 3) and 50 ng p132^{SEP1} (lane 4); 5 through 8, Western blot analysis of 10 μg of crude cell extracts of strain WDHY152 (sep1Δ/sep1Δ) vegetative cells (lane 5), WDHY150 (SEP1/SEP1) vegetative cells (lane 6), WDHY152 (sep1Δ/sep1Δ) pachytene cells (lane 7), and WDHY150 (SEP1/SEP1) pachytene cells (lane 8). The pachytene cells were from a 5-h time point of a meiotic time course in which the presence of synaptonemal complex structures had been confirmed by electron microscopic analysis as described previously (3). Lanes 1 through 4 come from one gel in which one half was stained with Coomassie blue and one half was transferred to nitrocellulose. Lanes 5 through 8 come from an independent gel. Size markers are indicated and were, from top to bottom, myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), BSA (66 kDa), and ovalbumin (45 kDa), each 0.5 μg per lane.

FIG. 2. Sep1 protein levels in S. cerevisiae during meiosis. (A) FACS analysis to monitor the progress through meiotic prophase, using strain RKY1154. (B) Relative amounts of Sep1 during meiosis. Closed circles, average of three independent meiotic time course with strain RKY1154 (a/α); open squares, average of two experiments with the α/α control strains (WDHY167 and WDHY193).

no bands were detected from extracts of an sep1 null mutant (lane 5). A similar specificity was observed for extracts from meiotic S. cerevisiae (lanes 7 and 8), indicating that no cross-reacting protein species were induced during meiosis.

Amount of Sep1 in S. cerevisiae cells. The total amount of Sep1 in S. cerevisiae cells was quantitated by Western blotting of crude cell extracts. Each blot contained a standard curve with purified Sep1 for calibration and quantitation. The data summarized in Table 2 show that Sep1 is an abundant protein, making up ~0.2% of the total soluble protein. This relative amount was constant in haploid, diploid, and tetraploid cells. We calculated 78,000 molecules of Sep1 per diploid cell. In heterozygous diploid cells, the amount of Sep1 was 50 to 70% of that in wild-type diploids (Table 2). Similar yields of Sep1 were obtained from total extracts and from soluble protein fractions, indicating that Sep1 was soluble.

Regulation of Sep1. To learn more about the possible cellular function of Sep1, we analyzed whether the protein level is regulated in the cell. For the meiotic analysis, we used the rapidly sporulating S. cerevisiae strain SK-1, in which synchronous meiotic time course experiments have been well established (48). The results of this analysis are summarized in Fig. 2. Figure 2A shows an example of a synchronous meiotic time course as monitored by FACS analysis. The bulk of premeiotic DNA replication was accomplished by 270 min as found previously (48, 62). The loss of signal beginning at 6 h and very
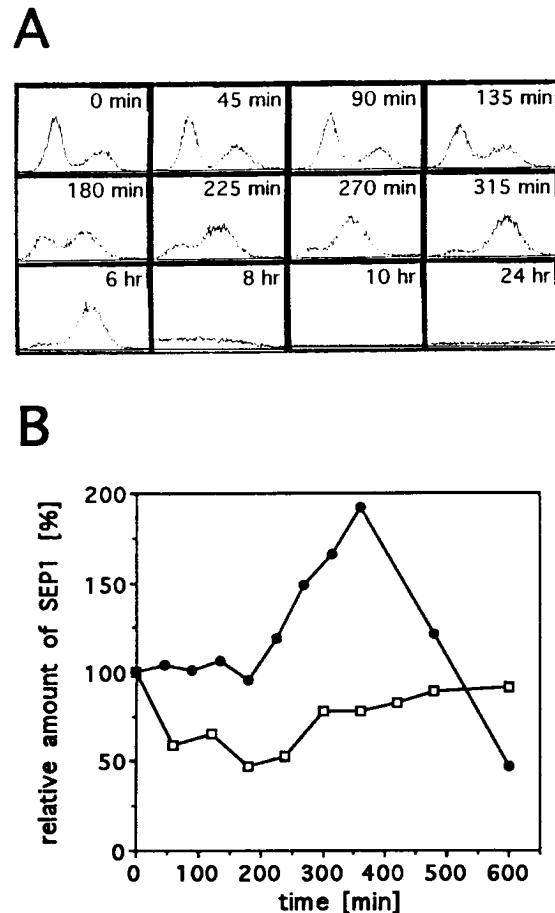
evident at 10 h signals spore formation as spores brightly stain with propidium iodide. Three independent time courses were performed with the SK-1-derived strain RKY1154, and sporulation was between 91 and 95%. Figure 2B shows an average twofold increase in relative amounts of Sep1 protein level during meiosis. The nonsporulating α/α control strain WDHY167 under the same conditions showed a twofold decrease of Sep1 immediately after shift (Fig. 2B), suggesting that the increase of Sep1 observed in wild-type cells was meiosis specific. A lower-molecular-weight form of Sep1 was apparent in the meiotic time course. It is likely to be a proteolytic

TABLE 2. Amounts of Sep1 in extracts of S. cerevisiae cells[a]

Strain	Ploidy	Molecules of Sep1/cell		% of soluble protein	
		Exponential phase	Stationary phase	Exponential phase	Stationary phase
Wild type (RKY1154)	2n	78,000 ± 24,000 (11)	63,000 ± 21,000 (5)	0.30 ± 0.09 (4)	0.22 ± 0.02 (3)
Wild type (NKY211)	1n	29,000 ± 12,000 (3)	50,000 ± 14,000 (3)	0.21 (1)	0.34 (1)
SEP1/sep1 (RKY1303)	2n	41,000 ± 12,000 (3)	46,000 ± 16,000 (3)	0.15 (1)	0.17 (1)
Wild type (LDY1)	4n	138,000 ± 21,000 (4)	105,000 ± 7000 (4)	0.21 ± 0.03 (4)	0.19 ± 0.02 (4)

[a] Total extracts and total soluble protein were prepared as described in Materials and Methods and were used to determine the absolute (molecules per cell) and relative (percentage of soluble protein) amounts of Sep1. Data are given as means ± 1 standard deviation where appropriate. The number of independent determinations is given in parentheses.

artifact of the native protein, since mixing extracts with a source of intact Sep1 led to a similar degradation of the intact Sep1 (data not shown). Sep1 was also detected in extracts of spores (data not shown).

Many genes show cell cycle-specific regulation, often reflecting when the gene product is required. We used α-factor arrest-release experiments to synchronize cells of strain L42 in their mitotic cell cycle. Control Northern (RNA) blot analysis showed the typical cycling for histone *HTA1* (18) and *RPA1* mRNAs (5). However, Sep1 RNA and protein levels did not change significantly. In addition, no significant differences in Sep1 levels were observed in various *cdc* mutant strain cells arrested at various points during the cell cycle (data not shown). Therefore, we conclude that Sep1 protein levels are unlikely to be regulated during the *S. cerevisiae* cell cycle.

S. cerevisiae cells lacking Sep1 display a delay in return to growth following exposure to DNA-damaging agents such as UV irradiation or methyl methane sulfonate (62). Under a variety of conditions (see Materials and Methods), no changes in the levels of Sep1 could be observed after treatment of the cells with UV or methyl methane sulfonate (data not shown).

Intracellular localization of Sep1. Sep1 is an abundant protein in *S. cerevisiae*. Nevertheless, the MAbs did not detect the protein in indirect immunofluorescence experiments. Consequently, we raised polyclonal antibodies specific for Sep1 and affinity purified the antiserum by using purified Sep1. The resulting antibody preparation was highly specific for Sep1 in Western blots of *S. cerevisiae* whole-cell extracts (data not shown). This affinity-purified anti-Sep1 antiserum was used for indirect immunofluorescence of whole *S. cerevisiae* cells. As seen in Fig. 3, the antibody was specific for Sep1 in fixed cells: a strong signal was observed in wild-type cells compared with a strain deficient for Sep1 (compare Fig. 3C and F). Sep1 was detected in the cytoplasm of wild-type cells and appeared to be excluded from the vacuole and nucleus (see Fig. 5C). This staining pattern was generally similar to that of the cytoplasmic ribosomal protein L3 (Fig. 3I). The fixation of these cells did not preclude staining nuclear proteins, since an antibody specific for Nsr1, a nucleolar protein, showed the expected nuclear staining (Fig. 3L). The localization of Sep1 did not appear to change during the cell cycle. The cells shown in Fig. 3 were actively growing and represented various stages of the cell cycle; however, the fluorescence signal for Sep1 remained cytoplasmic in all cases. Sep1 also appeared to be excluded from the nucleus in cells overexpressing the protein (data not shown).

To address the localization of Sep1 during meiosis, a diploid strain (RKY1308) was sporulated. This strain is in the SK-1 background, which displays rapid synchronous and efficient sporulation. Samples were taken at 4 and 6 h after transfer to sporulation medium, fixed, and prepared for indirect immunofluorescence as described for mitotic cells. In the time course presented, the wild-type culture was approximately 50 and 75% sporulated at 8 and 9.5 h, respectively, judged by the appearance of tetrads. After 24 h, the culture was >90% sporulated. Figure 4 shows Nomarski, DAPI, and indirect immunofluorescence images of wild-type cells. At 4 h, Sep1 appeared to be predominantly in the cytoplasm, with the greatest signal in the nuclear periphery. At 6 h, the staining for Sep1 was variable: it appeared that the antibodies did not penetrate all nascent tetrads equally, probably because the spore walls of the more developed tetrads did not allow passage of the antibody. At this time point, very few mature tetrads were observed by light microscopy, although the images in Fig. 4 show clearly that spores were forming. When Sep1 staining was observed in the nascent tetrads, Sep1 appeared to be packaged into the spores,

but staining was diminished in the region where the DNA was localized. At 0 h, the wild-type culture showed Sep1 staining similar to that observed in vegetative cells (data not shown). Cells of strain RKY1957 (*sep1Δ/sep1Δ*) at 0, 4, and 6 h in sporulation medium showed only a faint background signal similar to that shown in Fig. 3F (data not shown). In this experiment, RKY1957 showed no sporulation at 9.5 h and 9% sporulation after 24 h.

In the indirect immunofluorescence experiments, it was possible that there was a considerable fraction of Sep1 present in the nucleus of vegetative cells but that the protein in the nucleus was specifically not accessible to the antibody. To address this, we carried out cell fractionation and then subjected the various fractions to Western blot analysis in which the proteins would be denatured and epitopes that would be inaccessible in the folded protein should be revealed. Figure 5 shows the results from such an experiment. Whole-cell extracts and cytoplasmic and nuclear fractions were fractionated by SDS-PAGE. Sep1 and marker proteins were visualized by Western blotting, and the amounts of the various proteins were quantitated. In this experiment, topoisomerase II, a nuclear protein, was enriched approximately 16-fold in the nuclear fraction compared with the cytoplasmic fraction. Sep1 showed an enrichment in the nuclear fraction of 1.5-fold. The ribosomal protein L3, used as a cytoplasmic marker, was depleted fourfold in the nuclear fraction relative to the cytoplasmic fraction. Similar results were obtained in an independent experiment and with glucose-6-phosphate dehydrogenase as a cytoplasmic marker (data not shown). Comparison of the distribution of Sep1 with that of L3 and topoisomerase II indicated that 90 to 93% of the Sep1 in the crude extract was fractionating with the cytoplasmic proteins. Compared with the ribosomal protein L3, for which approximately 1.5% was found in the nuclear fraction, 7 to 10% of the total Sep1 was present in the nuclear fraction, suggesting a tighter association with the nucleus. Indirect immunofluorescence of the Sep1 present in the nuclear fraction showed perinuclear staining (data not shown). Thus, it is likely that most of the Sep1 present in the nuclear fractions that was detected by Western blotting was associated with the nuclear periphery. These results are consistent with the result obtained by indirect immunofluorescence that the bulk of Sep1 is cytoplasmic.

Proteins related to Sep1 in other species. The anti-Sep1 antibodies allowed us to examine extracts of various species for proteins that might be related to *S. cerevisiae* Sep1. As shown in Fig. 6, we detected various, mostly high-molecular-weight proteins from extracts of highly divergent organisms. A doublet with an M_r of ~140,000 was evident in extracts of the fission yeast *Schizosaccharomyces pombe* (Fig. 6, lane 2). The cross-reaction in *S. pombe* extracts was rather faint, and only a single MAb recognized this band. However, it was demonstrated in an independent study that this cross-reactive material represents the *S. pombe* homolog to Sep1 (30). Two cross-reactive bands with M_rs of ~170,000 (major band) and ~140,000 (minor band) were detected in *Drosophila melanogaster* embryo extracts (lane 3). High-molecular-weight signals were also obtained in *Xenopus laevis* germinal vesicles (M_r of ~150,000; lane 4) and in extracts from whole oocytes (M_r of ~120,000; lane 5). A variety of bands with M_rs of ~116,000, 62,000, and 54,000 were detected in mouse cell extracts (lane 6). The significance of the cross-reacting high-molecular-weight (>100,000) bands in *Xenopus* and mouse extracts is supported by the finding that this material was recognized by multiple MAbs specific for different epitopes of Sep1 (data not shown). No cross-reactive material was detected in extracts from cauliflower (data not shown).
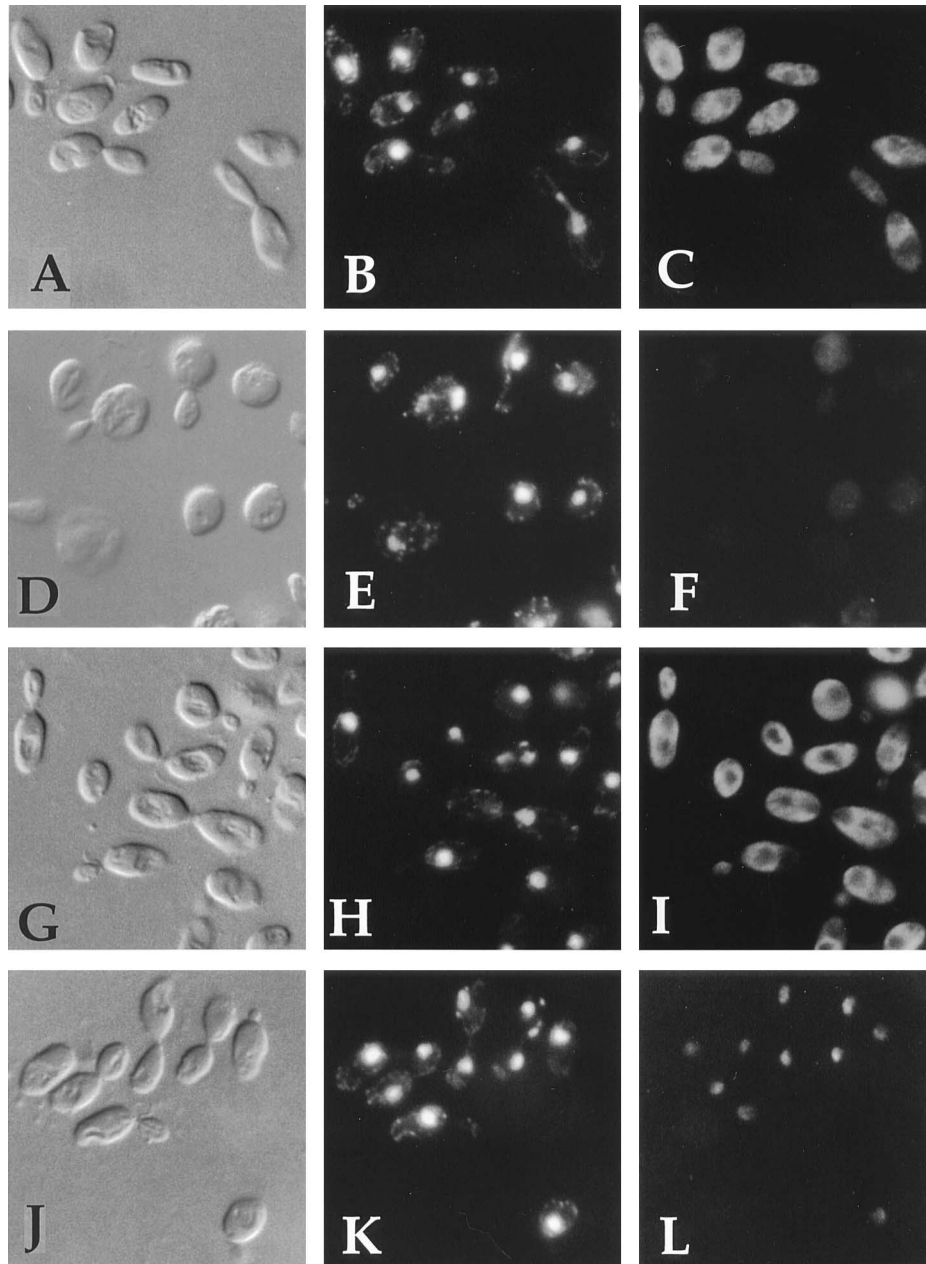
FIG. 3. Indirect immunofluorescence of whole mitotic cells. A log-phase culture was fixed and prepared for indirect immunofluorescence as described in the text. (A through C and G through L) Strain RKY1308 (wild type); (D through F) strain RKY1957 (*sep1Δ/sep1Δ*); (A, D, G, and J) Nomarski optics; (B, E, H, and K) DAPI staining; (C and F) indirect immunofluorescence using an affinity-purified anti-Sep1 polyclonal antibody at a 1:120 dilution; (I) indirect immunofluorescence using an anti-ribosomal protein L3 MAb diluted 1:2,000; (L) indirect immunofluorescence using a MAb specific for Nsr1 diluted to 2.5 μg/ml. Secondary antibodies were tetramethyl rhodamine isothiocyanate-conjugated goat anti-rabbit at 1.5 μg/ml and Cy3-conjugated donkey anti-mouse at 1.5 μg/ml.

DISCUSSION

Regulation and localization of Sep1. Native Sep1 is a large protein with an M_r of 175,000. The M_r-132,000 species reported originally (39) arose from proteolysis of the native protein during purification and is not found in vivo. We note that the protease-sensitive COOH terminus lacking in the p132^{SEP1} species is not present in the *S. pombe* homolog of Sep1 (60) and that it appears to be dispensable for most in vitro functions, including exonuclease and strand exchange, and for at least some in vivo functions, including normal growth, sporulation, and response to nitrogen starvation (39, 49).

Sep1 is an abundant protein, present at about 80,000 molecules per diploid cell and comprising roughly 0.2% of the total cellular protein in vegetative cells. Protein levels were not significantly altered following exposure to DNA-damaging agents or at various stages of the cell cycle. In addition, Sep1 does not appear to be phosphorylated in vegetative *S. cerevisiae* cells (24), although other posttranslational modifications which may regulate Sep1 activity have not been ruled out. In meiosis, Sep1 protein levels increase about twofold. It has been demonstrated that a number of genes both essential and nonessential for meiosis are transcriptionally induced in meiosis (28).
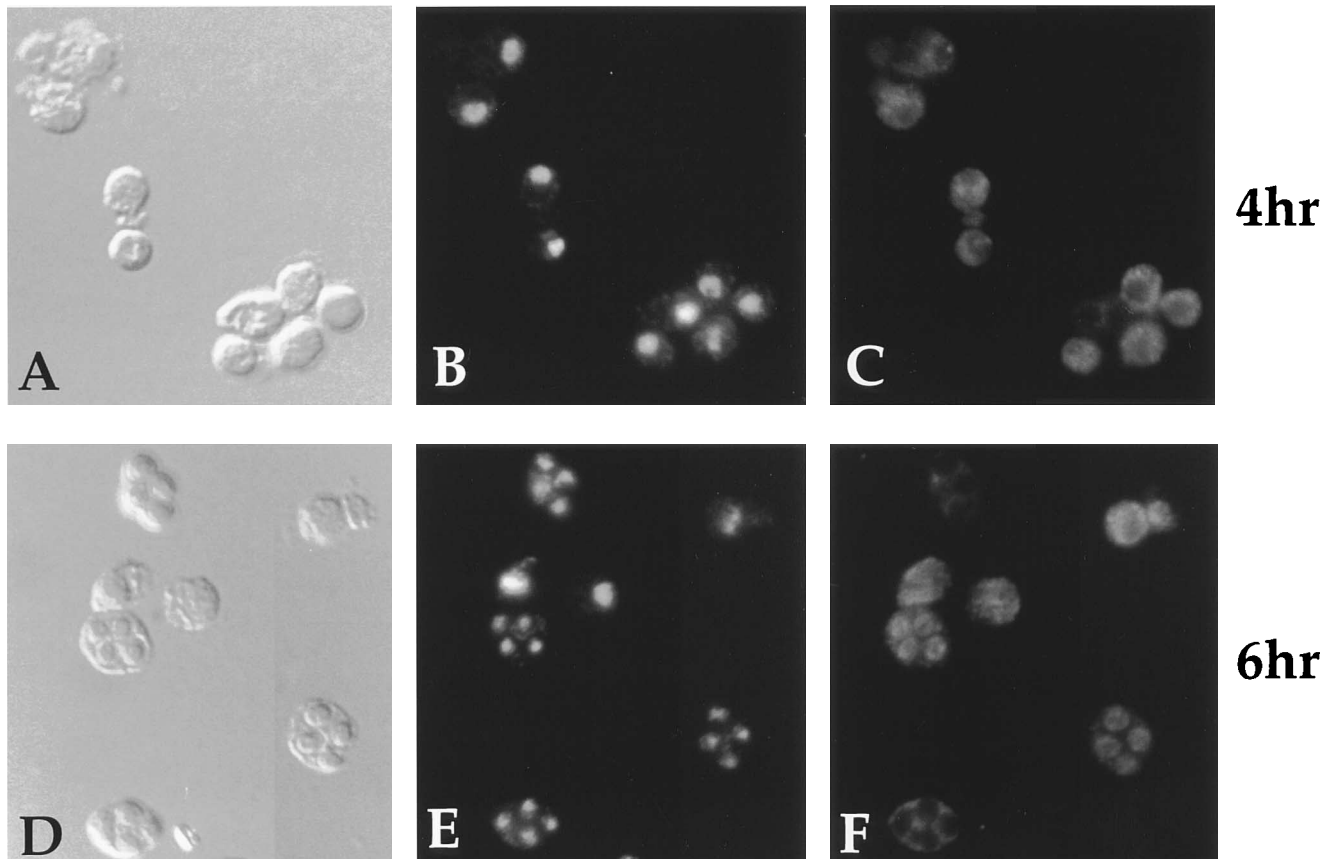
FIG. 4. Indirect immunofluorescence of meiotic cells. Samples from a synchronous meiotic culture of RKY1308 (wild type) were removed at 4 h (A through C) and 6 h (D through F) after transfer to sporulation medium. (A and D) Nomarski optics; (B and E) DAPI staining; (C and F) indirect immunofluorescence using an affinity-purified anti-Sep1 polyclonal antibody diluted 1:60.

However, it is not clear that the twofold increase of Sep1 protein levels during meiosis reflects a meiosis specific requirement for increased levels of Sep1. Lastly, in diploids heterozygous for *SEP1*, there was approximately half the wild-type level of Sep1, suggesting a lack of dosage compensation.

By a combination of indirect immunofluorescence and cell fractionation experiments, Sep1 was found to be predominantly localized to the cytoplasm. There was no apparent change in the localization of Sep1 during the mitotic cell cycle. A cytoplasmic localization was also observed by indirect immunofluorescence in meiotic cells. On the basis of previous characterizations of synchronous sporulation in SK-1 strains (48), the time points taken for meiotic cells were at pachytene (4 h) and early in spore development (6 h). In particular, at pachytene the Sep1 signal was greatest in the nuclear periphery.

Although the indirect immunofluorescence clearly indicated a cytoplasmic localization, it remained possible that a significant proportion of Sep1 actually was present in the nucleus but in a form not detected in the immunofluorescence studies. Since the nuclei could be stained with an antibody specific for the nucleolar protein Nsr1, the apparent exclusion of Sep1 from the nucleus probably was not an artifact of the cell preparation for immunofluorescence. In addition, we addressed this possibility by using cell fractionation. From the distribution of Sep1 relative to cytoplasmic and nuclear marker proteins, we conclude that at least 90% of Sep1 was fractionating with the cytoplasm. Compared with the cytoplasmic marker L3

protein, a greater relative amount of Sep1 was present in the nuclear fraction, although indirect immunofluorescence indicated that the Sep1 in the nuclear fraction was perinuclear. This is reminiscent of the perinuclear staining reported for an Sep1–β-galactosidase fusion protein which complemented a *kem1* (*sep1*) mutant (34). These data support the conclusion from indirect immunofluorescence studies of whole cells that the majority of Sep1 is found in the cytoplasm, although it is not possible to rule out the possibility that some small fraction is present in the nucleus.

Implications for the role of Sep1. The most straightforward interpretation of Sep1 function from the regulation and localization data is that Sep1 plays a general metabolic or structural role in the cytoplasm, where the vast majority of the protein is localized. This localization is generally compatible with a function in RNA turnover (see below) and/or in the cytoskeleton (35) and less compatible with a direct function in homologous recombination (see below) and/or DNA replication (37). Consistent with a cytoplasmic localization of Sep1 and a role in RNA turnover, *xrn1* (*sep1*) cells display a defect in processing 20S rRNA (56). The affected cleavage reaction is proposed to occur in the cytoplasm (65). It has been argued previously that Sep1 is an exoribonuclease important for mRNA turnover in the cytoplasm of *S. cerevisiae* (22). Consistent with this view, *xrn1* (*sep1*) mutants accumulate unadenylated uncapped transcripts. In addition, the exoribonuclease of Xrn1 (Sep1) is blocked by the presence of a m^7G cap structure (54), suggesting that Xrn1 (Sep1) acts at a late step in transcript turnover.
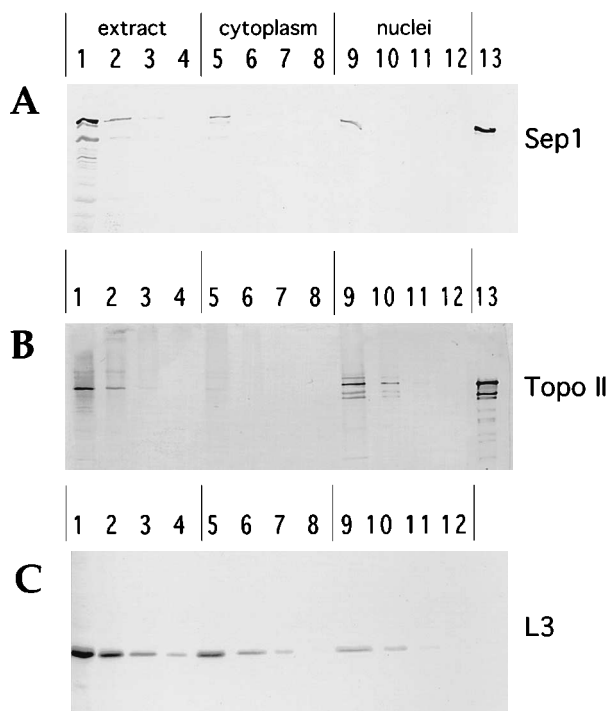
FIG. 5. Cell fractionation. Cells of a log-phase culture of RKY1308 were fractionated into whole-cell, nuclear, and cytoplasmic fractions. The fractions were analyzed by Western blotting, and the amounts of Sep1, topoisomerase II, and ribosomal protein L3 in each fraction were quantitated. (A [Sep1]) Lanes 1 through 4, 2, 0.5, 0.125, and 0.031 μl, respectively, of whole-cell extract; lanes 5 through 8, 5, 1.25, 0.31, and 0.078 μl, respectively, of cytoplasmic fraction; lanes 9 through 12, 12, 3, 0.75, and 0.188 μl, respectively, of nuclear fraction; lane 13, 20 ng of purified Sep1. An affinity-purified anti-Sep1 polyclonal antibody was used at a dilution of 1:1,000. (B [topoisomerase II]) Lanes as for panel A except lane 13 (10 ng of purified topoisomerase II). An anti-topoisomerase II polyclonal antibody was used at a dilution of 1:2,500. (C [ribosomal protein L3]) Lanes as for panel A except that lane 13 was omitted. Mouse anti-L3 (MAb 7.1) was used at a dilution of 1:5,000.

FIG. 6. Western blot analysis of extracts from various organisms with anti-Sep1 antibodies. Lanes: 1, 10 μg of cell extract of *S. cerevisiae*; 2, 50 μg of cell extract of *S. pombe*; 3, 13 μg of cell extract of *D. melanogaster* embryos (kindly supplied by M. Sander); 4, 87 μg of germinal vesicle extract of *X. laevis*; 5, 300 μg of oocyte extract of *X. laevis* (both kindly supplied by D. Carroll); 6, 150 μg of extract of mouse pre-B-cell line PD31 (kindly supplied by D. Weaver). Samples in lane 1, 4, and 5 were analyzed on one gel; all others were analyzed on different gels. M_r determinations were done with size markers run close to the sample to be analyzed. Dots indicate positions of bands. Size markers are indicated for orientation and were as described for Fig. 1.

A pathway of mRNA turnover in *S. cerevisiae* in which transcripts are deadenylated, subsequently decapped, and finally degraded by 5′-to-3′ exonucleases has recently been described (47). For certain rapidly degraded transcripts such as *MFα1*, degradation of the deadenylated and decapped transcripts is significantly slowed in an *xrn1* (*sep1*) mutant, providing evidence that Sep1 plays a role in this process. The uncapped and unadenylated transcripts that accumulate in *sep1* mutants appear to be retained in polysomes, suggesting that they are translated. It is possible that the pleiotropy of *sep1* mutants arises at least partially from a reduced rate of transcript turnover, leading to the expression of highly regulated genes by translation of uncapped mRNA at inappropriate times in the cell cycle. Aberrant protein levels have been reported in *xrn1* (*sep1*) mutants (43).

Sep1 appears to be the most abundant protein encoding a 5′-to-3′ exoribonuclease in *S. cerevisiae* (44), suggesting that it is an important but not essential enzyme for RNA turnover. A rough calculation can be made of the capacity of the pool of Sep1 in a cell for RNA turnover. Assuming a turnover number for single-stranded RNA of 400 to 700 nucleotides per min (24, 55) and approximately 40,000 molecules of Sep1 per haploid cell, there is sufficient enzyme to degrade one-half to one genome equivalent of RNA per min. We believe that the cytoplasmic localization, the high abundance, and the apparent lack of regulation are consistent with the notion that Sep1 is
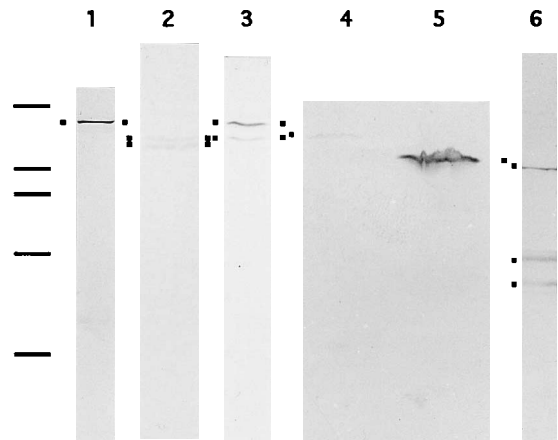
needed throughout the cell cycle for RNA degradation. A second and essential 5′-to-3′ exoribonuclease, exonuclease II, encoded by the *HKE1* gene, has been described in *S. cerevisiae* (33). Although exonuclease II appears much less abundant than Sep1, it appears to account for a similar amount of exoribonuclease activity as measured on poly(A) (33, 44). *HKE1* is also referred to as *RAT1* (1) and *TAP1* (9). A role for exonuclease II in transcript turnover has not been described.

Sep1 in DNA recombination. Sep1 was identified as a putative DNA recombination protein that catalyzed the formation of heteroduplex DNA from single-stranded and homologous linear duplex DNA molecules in vitro (10, 39). This reaction has been used extensively for the characterization of bacterial and bacteriophage recombination proteins (19, 41). Although the in vitro activities of Sep1 are consistent with an in vivo role in DNA recombination, other proteins that appear not to have a role in recombination have been shown to promote the formation of heteroduplex DNA in vitro. These include *S. pombe* fatty acid synthetase (31), *S. cerevisiae* translation elongation factor 3 (46), and *S. cerevisiae* transcription elongation factor SII (TFIIS) (8, 36, 58). It should be pointed out that while the gene identified as encoding the DNA strand transfer protein α was TFIIS, purified *S. cerevisiae* SII protein does not carry out strand exchange (8), raising questions as to whether the correct gene encoding DNA strand transfer protein α was cloned. Furthermore, purified *Drosophila* SII protein (15) and human TFIIS also do not appear to catalyze strand exchange (29). On the other hand, bacterial and bacteriophage proteins known to be required in recombination and which can replace RecA in certain recombination reactions (16, 38, 40, 41) catalyze DNA pairing in a mode similar to that of Sep1 (26). Furthermore, purified Sep1 protein has been shown to promote the formation of paranemic joints (7), the pairing of a single-stranded DNA with duplex DNA without the intertwining of strands. This reaction is thought to be highly specific for recombination proteins, supporting the hypothesis that Sep1 is a recombination protein (7, 41).

The meiotic recombination defects discovered so far in *sep1*

cells can be interpreted in several ways. The meiotic arrest of *sep1* mutants cannot be bypassed by *spo13*, *spo13 rad50*, or *spo11 spo13* mutations, suggesting that Sep1 could be involved in some type of *RAD50* epistasis group-independent recombination pathway. However, the proposal that Sep1 mediates meiotic chromosome pairing through the formation of G4 tetrastranded DNA seems unlikely since *sep1* null mutants do not display significant pairing defects (3, 63). The recombination defects observed could also be a pleiotropic consequence of another primary defect(s) such as RNA metabolism (see above) or microtubule function (35) or be due to the meiotic arrest (3, 62, 63). In this respect, yeast strains that sporulate efficiently appear to have high RNase levels, possibly suggesting a requirement for RNA turnover during sporulation (64). However, a pachytene arrest point similar to that observed in *sep1* mutants has been observed for the cell cycle mutation *cdc28* (52), and recombination is relatively normal in *cdc28* mutants subjected to pachytene arrest, indicating that pachytene arrest per se does not cause a recombination deficiency (63, 66). On the basis of all available data, we cannot rule out the possibility that Sep1 plays a direct role in homologous recombination.

Sep1 is conserved in evolution. Sep1 appears to be conserved throughout evolution in eukaryotes. A homolog has been identified in *S. pombe* (30, 60), a yeast highly diverged from *S. cerevisiae* (53). Genes having homology to Sep1 have been identified in humans (13) and, using antibodies specific for Sep1, immunocross-reactive species have been identified in extracts of mouse cells, *Drosophila* embryos, and frog oocytes. It is possible that not all of the cross-reactive bands represent homologs of Sep1, but the high-molecular-weight bands in *Xenopus* and mouse cells, cross-reacted with several MAbs recognizing different epitopes of Sep1, suggesting that they are homologs of Sep1. Understanding the role of Sep1 in *S. cerevisiae* will help in understanding the role of this highly conserved protein in higher eukaryotic cells.

REFERENCES

1. **Amberg, D. C., L. A. Goldstein, and C. N. Cole.** 1992. Isolation and characterization of *RAT1*: an essential gene of Saccharomyces cerevisiae required for the efficient nucleocytoplasmic trafficking of mRNA. Genes Dev. **6:**1173–1189.
2. **Aris, J. P., and G. Blobel.** 1991. Isolation of yeast nuclei. Methods Enzymol. **194:**735–749.
3. **Bähler, J., G. Hagens, G. Holzinger, H. Scherthan, and W.-D. Heyer.** 1994. *Saccharomyces cerevisiae* cells lacking the homologous pairing protein p175^*SEP1* arrest at pachytene during meiotic prophase. Chromosoma **103:** 129–141.
4. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
5. **Brill, S. J., and B. Stillman.** 1991. Replication factor-A from *Saccharomyces cerevisiae* is encoded by three essential genes coordinately expressed at S phase. Genes Dev. **5:**1589–1600.
6. **Chao, S., and J. Woolford. (Carnegie Mellon University).** 1994. Personal communication.
7. **Chen, J., R. Kanaar, and N. R. Cozzarelli.** 1994. The Sep1 strand exchange protein from *Saccharomyces cerevisiae* promotes a paranemic joint between homologous DNA molecules. Genes Dev. **8:**1356–1365.
8. **Clark, A. B., C. C. Dykstra, and A. Sugino.** 1991. Isolation, DNA sequence, and regulation of a *Saccharomyces cerevisiae* gene that encodes DNA strand transfer protein alpha. Mol. Cell. Biol. **11:**2576–2582.
9. **Di Segni, G., B. L. McConaughy, R. A. Shapiro, T. L. Aldrich, and B. D. Hall.** 1993. *TAP1*, a yeast gene that activates the expression of a tRNA gene with a defective internal promoter. Mol. Cell. Biol. **13:**3424–3433.
10. **Dykstra, C. C., R. K. Hamatake, and A. Sugino.** 1990. DNA strand transfer protein β from yeast mitotic cells differs from strand transfer protein α from meiotic cells. J. Biol. Chem. **265:**10968–10973.
11. **Dykstra, C. C., K. Kitada, A. B. Clark, R. K. Hamatake, and A. Sugino.** 1991. Cloning and characterization of *DST2*, the gene for DNA strand transfer protein β from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:**2583–2592.
12. **Elledge, S. J., and R. W. Davis.** 1989. DNA damage induction of ribonucleotide reductase. Mol. Cell. Biol. **9:**4932–4940.
13. **Fishel, R. (University of Vermont, Burlington).** 1994. Personal communication.
14. **Goto, T., and J. C. Wang.** 1984. Yeast DNA topoisomerase II is encoded by a single-copy, essential gene. Cell **36:**1073–1080.
15. **Guo, H., D. H. Price, A. W. Johnson, and R. D. Kolodner.** Unpublished results.
16. **Hall, S. D., and R. D. Kolodner.** 1994. Homologous pairing and strand exchange promoted by the *Escherichia coli* RecT protein. Proc. Natl. Acad. Sci. USA **91:**3205–3209.
17. **Henry, Y., H. Wood, J. P. Morrissey, E. Petfalski, S. Kearsey, and D. Tollervey.** 1994. The 5′ end of yeast 5.8S rRNA is generated by exonucleases from an upstream cleavage site. EMBO J. **13:**2452–2463.
18. **Hereford, L. M., S. Bromley, and M. A. Osley.** 1982. Periodic transcription of yeast histone genes. Cell **30:**305–310.
19. **Heyer, W.-D.** 1994. The search for the right partner: homologous pairing and DNA strand exchange proteins in eukaryotes. Experientia **50:**223–233.
20. **Heyer, W.-D., D. H. Evans, and R. D. Kolodner.** 1988. Renaturation of DNA by a *Saccharomyces cerevisiae* protein that catalyzes homologous pairing and strand exchange. J. Biol. Chem. **263:**15189–15195.
21. **Heyer, W.-D., M. R. S. Rao, L. F. Erdile, T. J. Kelly, and R. D. Kolodner.** 1990. An essential *Saccharomyces cerevisiae* single-stranded DNA binding protein is homologous to the large subunit of human RP-A. EMBO J. **9:**2321–2329.
22. **Hsu, C. L., and A. Stevens.** 1993. Yeast cells lacking 5′→3′ exoribonuclease 1 contain mRNA species that are poly(A) deficient and partially lack the 5′ cap structure. Mol. Cell. Biol. **13:**4826–4835.
23. **Hutter, K.-J., and H. E. Eipel.** 1978. Microbial determinations by flow cytometry. J. Gen. Microbiol. **113:**369–375.
24. **Johnson, A. W., and R. D. Kolodner.** Unpublished results.
25. **Johnson, A. W., and R. D. Kolodner.** 1991. Strand exchange protein 1 from *Saccharomyces cerevisiae*. A novel multifunctional protein that contains DNA strand exchange and exonuclease activities. J. Biol. Chem. **266:**14046–14054.
26. **Johnson, A. W., and R. D. Kolodner.** 1994. Characterization of the interaction of *Saccharomyces cerevisiae* strand exchange protein 1 with DNA. J. Biol. Chem. **269:**3673–3681.
27. **Jones, E. W.** 1991. Tackling the protease problem in *Saccharomyces cerevisiae*. Methods Enzymol. **194:**428–453.
28. **Kaback, D. B., and R. Feldberg.** 1985. *Saccharomyces cerevisiae* exhibits a sporulation-specific temporal pattern of transcript accumulation. Mol. Cell. Biol. **5:**751–761.
29. **Kane, C. M. (University of California, Berkeley).** 1994. Personal communication.
30. **Käslin, E., and W.-D. Heyer.** 1994. A multi-functional exonuclease from vegetative *Schizosaccharomyces pombe* cells exhibiting *in vitro* strand exchange activity. J. Biol. Chem. **269:**14094–14102.
31. **Käslin, E., and W.-D. Heyer.** 1994. *Schizosaccharomyces pombe* fatty acid synthase mediates DNA strand exchange *in vitro*. J. Biol. Chem. **269:**14103–14110.
32. **Kearsey, S., and D. Kipling.** 1991. Recombination and RNA processing: a common strand? Trends Cell. Biol. **1:**110–112.
33. **Kenna, M., A. Stevens, M. McCammon, and M. G. Douglas.** 1993. An essential yeast gene with homology to the exonuclease-encoding *XRN1/KEM1* gene also encodes a protein with exoribonuclease activity. Mol. Cell. Biol. **13:**341–350.
34. **Kim, J.** 1988. Genes controlling conjugation and mitotic cell division in yeast *Saccharomyces cerevisiae*. Ph.D. thesis. Massachusetts Institute of Technology, Cambridge.

35. **Kim, J., P. O. Ljungdahl, and G. R. Fink.** 1990. *kem* mutations affect nuclear fusion in *Saccharomyces cerevisiae*. Genetics **126:**799–812.
36. **Kipling, D., and S. E. Kearsey.** 1991. TFIIS and strand-transfer proteins. Nature (London) **353:**509.
37. **Kipling, D., C. Tambini, and S. E. Kearsey.** 1991. *rar* mutations which increase artificial chromosome stability in Saccharomyces cerevisiae identify transcription and recombination proteins. Nucleic Acids Res. **19:**1385–1391.
38. **Kmiec, E., and W. K. Holloman.** 1981. β protein of bacteriophage λ promotes renaturation of DNA. J. Biol. Chem. **256:**12636–12639.
39. **Kolodner, R. D., D. H. Evans, and P. T. Morrison.** 1987. Purification and characterization of an activity from *Saccharomyces cerevisiae* that catalyzes homologous pairing and strand exchange. Proc. Natl. Acad. Sci. USA **84:**5560–5564.
40. **Kolodner, R., S. D. Hall, and C. Luisi-DeLuca.** 1994. Homologous pairing proteins encoded by the Escherichia coli recE and recT genes. Mol. Microbiol. **11:**23–30.
41. **Kowalczykowski, S. C., and A. K. Eggleston.** 1994. Homologous pairing and DNA strand exchange proteins. Annu. Rev. Biochem. **63:**991–1043.
42. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
43. **Larimer, F. W., C. L. Hsu, M. K. Maupin, and A. Stevens.** 1992. Characterization of the XRN1 gene encoding a 5′→3′ exoribonuclease: sequence data and analysis of disparate protein and mRNA levels of gene-disrupted yeast cells. Gene **120:**51–57.
44. **Larimer, F. W., and A. Stevens.** 1990. Disruption of the gene *XRN1*, coding for a 5′-3′ exoribonuclease, restricts yeast cell growth. Gene **95:**85–90.
45. **Liu, Z., and W. Gilbert.** 1994. The yeast *KEM1* gene encodes a nuclease specific for G4 tetraplex DNA: implication of in vivo functions for this novel DNA substrate. Cell **77:**1083–1092.
46. **McEntee, K. (University of California, Los Angeles).** 1994. Personal communication.
47. **Muhlrad, D., and R. Parker.** 1992. Mutations affecting stability and deadenylation of the yeast MFA2 transcript. Genes Dev. **6:**2100–2111.
48. **Padmore, R., L. Cao, and N. Kleckner.** 1991. Temporal comparison of recombination and synaptonemal complex formation during meiosis in *S. cerevisiae*. Cell **66:**1239–1256.
49. **Page, A., A. W. Johnson, and R. D. Kolodner.** Unpublished results.
50. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1982. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
51. **Sherwood, P. W., and M. A. Osley.** 1991. Histone regulatory (*hir*) mutations suppress δ insertion alleles in *Saccharomyces cerevisiae*. Genetics **128:**729–738.
52. **Shuster, E. O., and B. Byers.** 1989. Pachytene arrest and other meiotic effects of the Start mutations in Saccharomyces cerevisiae. Genetics **123:**29–43.
53. **Sipiczki, M.** 1989. Taxonomy and phylogenesis, p. 431–452. *In* A. Nasim, P. Young, and B. F. Johnson (ed.), Molecular biology of the fission yeast. Academic Press, Inc., San Diego, Calif.
54. **Stevens, A.** 1978. An exoribonuclease from *Saccharomyces cerevisiae*: effect of modification of the 5′ end groups on the hydrolysis of substrates to 5′-nucleotides. Biochem. Biophys. Res. Commun. **81:**656–661.
55. **Stevens, A.** 1980. Purification and characterization of a *Saccharomyces cerevisiae* exoribonuclease which yields 5′-mononucleotides by a 5′ → 3′ mode of hydrolysis. J. Biol. Chem. **255:**3080–3085.
56. **Stevens, A., C. L. Hsu, K. R. Isham, and F. W. Larimer.** 1991. Fragments of the internal transcribed spacer 1 of pre-rRNA accumulate in *Saccharomyces cerevisiae* lacking 5′→3′ exoribonuclease 1. J. Bacteriol. **173:**7024–7028.
57. **Sugawara, N., and J. Haber (Brandeis University).** 1994. Personal communication.
58. **Sugino, A., J. Nitiss, and M. A. Resnick.** 1988. ATP-independent DNA strand transfer catalyzed by protein(s) from meiotic cells of the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **85:**3683–3687.
59. **Symington, L. S., L. M. Fogarty, and R. Kolodner.** 1983. Genetic recombination of homologous plasmids catalyzed by cell-free extracts of *Saccharomyces cerevisiae*. Cell **35:**805–813.
60. **Szankasi, P., and G. R. Smith (Hutchinson Cancer Center).** 1994. Personal communication.
61. **Taggart, R. T., and I. M. Samloff.** 1983. Stable antibody-producing murine hybridomas. Science **219:**1228–1230.
62. **Tishkoff, D., A. W. Johnson, and R. D. Kolodner.** 1991. Molecular and genetic analysis of the gene encoding the *Saccharomyces cerevisiae* strand exchange protein SEP1. Mol. Cell. Biol. **11:**2593–2608.
63. **Tishkoff, D. X., B. Rockmill, G. S. Roeder, and R. D. Kolodner.** 1995. The *sep1* mutant of *Saccharomyces cerevisiae* arrests in pachytene and is deficient in meiotic recombination. Genetics **139:**495–509.
64. **Tsuboi, M.** 1976. Correlation among turnover of nucleic acids, ribonuclease activity and sporulation ability of *Saccharomyces cerevisiae*. Arch. Microbiol. **111:**13–19.
65. **Udem, S. A., and J. R. Warner.** 1973. The cytoplasmic maturation of a ribosomal precursor ribonucleic acid in yeast. J. Biol. Chem. **248:**1412–1416.
66. **Xu, L., and N. Kleckner (Harvard University).** 1994. Personal communication.