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Biochemistry

Isolation of the gene encoding yeast single-stranded nucleic acid binding protein 1

(unwinding protein/gene disruption/λgt11)

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ABSTRACT **A yeast gene encoding SSB-1, a single-stranded nucleic acid binding protein, has been isolated by screening a λgt11 genomic DNA library. The gene is located on a 1.84-kilobase chromosomal *Bgl* II–*Bam*HI fragment. Yeast strains carrying the high-copy-number vector YEp24 with an *SSB1* gene insert overproduce SSB-1 3-fold and SSB-1 mRNA 10-fold. A typical haploid cell contains about 20,000 molecules of SSB-1; thus, the cells can tolerate up to 60,000 copies. Yeast SSB-1 was expressed in *Escherichia coli* cells by using a phage T7 expression system. Spores containing the gene disrupted at a point within the coding sequence germinate and grow normally; thus, the gene is not essential. Protein blots show that no SSB-1 or novel immunologically related species that might retain SSB-1 activity are present in cells containing the disrupted *SSB1* genes. Southern analysis and protein blots suggest the presence in yeast of a second, related, but nonidentical gene and two immunologically related proteins of 55 kDa and 75 kDa.**

Studies on prokaryotic cells have indicated that single-stranded nucleic acid binding proteins (SSBs) are involved in multiple aspects of DNA metabolism such as DNA replication, recombination, and repair; thus, they are essential for the viability of the cell or phage (1, 2). In *Escherichia coli*, a single species of SSB functions in all these processes. In contrast, numerous species of cellular proteins that bind tightly to single-stranded DNA and RNA have been described in eukaryotes, and they fall into several classes.

One class, having molecular masses between 20 and 30 kDa, is represented by the 24-kDa calf thymus UP1 and the mouse HD-1 proteins. Recent evidence has revealed remarkable amino acid sequence conservation between the mouse and calf proteins (3), suggesting an important role for these proteins in DNA metabolism. These proteins lower the melting temperature of nucleic acids and stimulate their respective DNA polymerase α enzymes on single-stranded DNA templates. Although this has been used to suggest that they may play a role in DNA replication, there is no direct evidence for this. Sapp *et al.* (4) have described two larger calf thymus SSBs, 48 kDa and 61 kDa, that simulate DNA polymerase α and that elicit antibody that crossreacts with a 25-kDa protein likely to be UP1. It is not clear whether these various forms of related proteins are encoded by the same gene and arise by RNA or protein processing or if they are encoded by different members of a gene family. A second class of binding protein, from Novikoff hepatoma cells, stimulates DNA polymerase β and not polymerase α (5). The third class of SSB, which stimulates both DNA polymerase α and β, has been shown to be different from UP1 and HD-1 and has been identified as HMG1 (high mobility group protein 1) (6). The existence of more than one type of SSB that stimulates polymerase raises the question of which, if any, is analogous to the unique prokaryotic SSBs required for replication and recombination and that stimulate polymerases by specific protein–protein interactions. A fourth class of SSB, found in viral systems, is the 59-kDa adenovirus DNA binding protein, which is required for viral gene expression as well as for DNA replication both *in vivo* and *in vitro* (7).

There is also a fifth important class of eukaryotic SSB with molecular weights ranging from 30,000 to 40,000 (8–11). These proteins bind to single-stranded RNA as well as to single-stranded DNA and may be components of heterogeneous nuclear RNA particles. It is interesting that antibodies to the calf thymus SSBs of the first class described above (20–30 kDa) may crossreact with these putative heterogeneous nuclear RNA proteins (10), since this also may suggest that the SSBs are encoded by a family of related genes or are related to each other by processing. Considering the complexity of eukaryotes, the single species of SSB that carries out multiple functions in bacteria may have evolved into several discrete proteins in higher cells. However, there is no compelling evidence that any one of these has functions equivalent to the *E. coli* SSB.

To determine the functions and the interrelationships of the various eukaryotic SSBs, we have chosen yeast as a model system in which we can take a combined biochemical and genetic approach. A set of SSBs has been purified and characterized biochemically and immunologically (12). One of these, SSB-1, stimulates DNA polymerase I, and antibodies against SSB-1 inhibit *in vitro* replication. In order to assess the *in vivo* role of this protein, the gene encoding SSB-1 has been isolated. Here, the initial characterization of the gene, overproduction in yeast and *E. coli*, and the results of disrupting the gene *in vivo* are described.

MATERIALS AND METHODS

Strains and Media. *Saccharomyces cerevisiae* strain YM214 (*α his3Δ200 lys2-801 ade1-101 ura3-52*) from Mark Johnston (Washington University, St. Louis, MO) was used for protein blotting studies. Strain SS111 (*MATa, trp1-289, ura3-52, his3-532, ade2-10, gal2*) from S. Scherer (Department of Biology, California Institute of Technology) was used for RNA blotting experiments. The diploid strain JLC3 (YM214 × YM259: *MATa/α his3Δ200/his3Δ200 lys2-801/+ tyr1/+ ade2-101/ade2-101 ura3-52/ura3-52*) and strain SEY2101/SEY2102 (*MATa/α, leu2-3,112/leu2-3,112 ura3-52/ura3-52 Δsuc2/Δsuc2 ADE2/ade2 HIS4/his4-519*) were used for gene-disruption experiments. *E. coli* strain Y1090 (*ΔlacU169 Δlon araD139 strA supF trpC22 Tn10*::pMc9) (13) was used for the λgt11 screening; strain K38 was the gift of S. Tabor and C. C. Richardson (Harvard Medical School,

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Abbreviations: SSB, single-stranded nucleic acid binding protein; URA$^+$, uracil prototrophy.

Boston) and was used for the expression of yeast gene for SSB-1, *SSB1*.

Reagents and Enzymes. ^{125}I-labeled protein A and [α-^{32}P]dTTP were obtained from Amersham. Restriction enzymes were purchased from either New England Biolabs or Bethesda Research Laboratories. T7 RNA polymerase was obtained from United States Biochemical (Cleveland, OH). Nonidet P-40 was purchased from Particle Data (Elmhurst, IL). Fetal calf serum was from Irvine Scientific. Prestained protein standards were purchased from Bethesda Research Laboratories.

Plasmids and Yeast Genomic Libraries. For gene disruption, the *Eco*RI–*Hpa* I restriction fragment within the *SSB1* gene (Fig. 1), with an *Xho* I linker inserted into it at the *Hae* III site, was cloned into YIp5 to give pGX6. pT7-a and pT7-b were constructed by inserting the 1.95-kb *Bgl* II fragment containing the *SSB1* gene (see Fig. 1) into the *Bam*HI restriction site of pT7-1 (14) in two orientations.

Phage λgt11 is described by Young and Davis (13), and the λgt11 genomic DNA library was a gift of M. Snyder (Stanford School of Medicine, Stanford, CA). The YEp24 library was the gift of D. Botstein (Massachusetts Institute of Technology, Cambridge, MA).

λgt11 Plaque Screening. This was carried out as described by Johnson *et al.* (15).

Hybridizations. *Plaque hybridizations.* Transfer of phage DNA to nitrocellulose filters (Schleicher and Schuell BA85) was performed as described by Benton and Davis (16). Hybridization and washing of the nitrocellulose replicas was as described by Maniatis *et al.* (17).

Colony hybridization. Approximately 10^4–10^5 cells were spread on L agar plates containing 100 μg of ampicillin per ml (150-mm plates) and incubated at 37°C. The resultant colonies were lifted onto nitrocellulose filters and amplified on plates containing 10 μg of chloramphenicol per ml. Cell lysis and hybridization to nitrocellulose filter was performed as described by Maniatis *et al.* (17).

Protein blotting. Protein samples were prepared as described (12) and electroblotted to nitrocellulose paper by using a Bio-Rad Trans-Blot cell as described by the manufacturer. The filter papers were incubated with ^{125}I-labeled protein A and washed as described (15). For estimating the number of SSB-1 molecules in cells, purified SSB-1 and a crude extract from 0.2×10^7 or 1×10^7 cells were run on 12.5% polyacrylamide gel. The number of cells was determined by (*i*) light scattering, where $A_{590} = 1.5$ is equal to 10^7 cells per ml, and (*ii*) counting of the viable cells on yeast/peptone/dextrose plates using a serial dilution.

RNA analysis. Poly(A) RNA was isolated from 1 liter of cells grown on SD medium without uracil as described by Domdey *et al.* (18).

RESULTS

Screening of Yeast Genomic Libraries. λgt11 is a phage expression vector that can synthesize fusion proteins between inserted protein coding sequences and β-galactosidase. A yeast genomic DNA library constructed in phage λgt11 (13, 19) was screened for the *SSB1* gene by using affinity-purified polyclonal antibodies against SSB-1 (12). Of 3×10^5 plaques screened, 12 positive clones were identified. The 2 that gave the strongest signals were shown to contain overlapping DNA sequences by restriction enzyme mapping (data not shown). Since λgt11 is a phage expression vector that was constructed with the expectation that inserted DNA would be expressed as a β-galactosidase fusion protein, we expected that only part of the *SSB1* gene would be linked to the *lacZ* region of λgt11. In order to obtain an intact yeast *SSB1* gene, a yeast DNA fragment was isolated from one of the positive λ clones and used as a hybridization probe to screen a second yeast genomic library prepared in the high-copy-number plasmid YEp24. Of 10^5 colonies screened, five positive signals, designated pSB1-1 to pSB1-5, with inserts ranging from 6.5 to 20 kb were obtained. A restriction map of pSB1-4 (6.5-kb insert) is shown in Fig. 1. As will be shown below, both pSB1-4 and, surprisingly, the λgt11 clone contain the entire *SSB1* coding sequence.

Detection of Overproduction of SSB-1 by Protein Blotting. One way to verify that the clones in the high-copy-number vector YEp24 carry the *SSB1* gene is to demonstrate overproduction due to increased gene dosage in yeast. The SSB-1 protein itself does not have any detectable enzyme activity, but the expression of the *SSB1* gene could be measured by immunoblotting. A protein blot showing the relative quantities of SSB-1 protein in crude extracts of strain YM214 and a transformant carrying plasmid pSB1-4 is shown in Fig. 2. When bands corresponding to SSB-1 were cut out of the nitrocellulose paper and the radioactivity was determined, the pSB1-4 plasmid-containing strain showed 3-fold higher levels of SSB-1 than did YM214. (RNA blotting shows a 10-fold overproduction of 1.2-kb RNA; data not shown.) Thus, plasmid pSB1-4 carries either the structural gene for

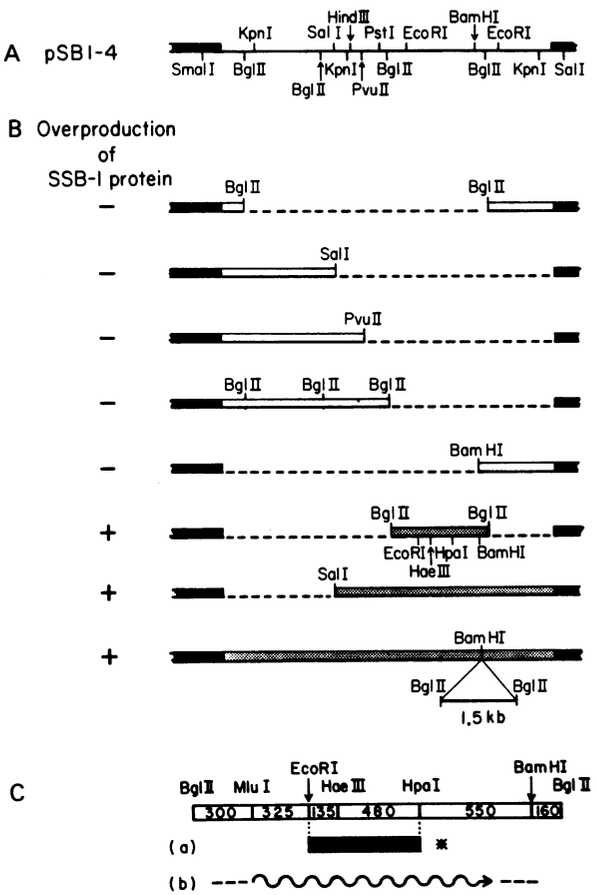

FIG. 1. Restriction map of the cloned region and of deletion and insertion mutations used in this study. (*A*) Restriction map of pSB1-4. Black bar, vector YEp24 DNA; solid line, yeast DNA fragment inserted in YEp24. (*B*) Plasmid pSB1-4 was digested with various restriction enzymes as shown in the figure. The resulting plasmids were transformed into yeast strain YM214. Open boxes indicate that the inserted DNA fragment does not overproduce SSB-1; stippled boxes indicate that the inserted DNA fragments overproduce SSB-1. (*C*) Restriction map of the *Bgl* II fragment of 1.95 kb containing the *SSB1* gene. The *Eco*RI–*Hpa* I fragment, an internal fragment of the *SSB1* gene that was cloned into integrating vector YIp5 for gene disruption experiments in Fig. 4, is shown. The direction of transcription and the approximate location of the mRNA transcript are indicated by the wavy line.

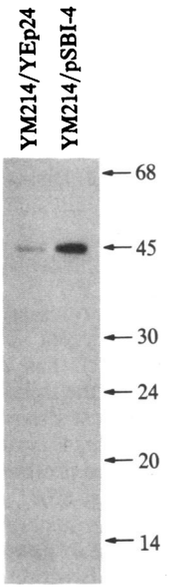

FIG. 2. Protein blot analysis of the SSB-1 protein in yeast cells containing the isolated gene on a high-copy-number plasmid. Strain YM214 was transformed with pSB1-4 (carrying the *SSB1* gene) or YEp24 (the control). Crude extract (5 μg) was electrophoresed in a 12.5% polyacrylamide gel and then transferred to nitrocellulose paper. Affinity-purified SSB-1 antibodies were used to locate the SSB-1 proteins. Antigen–antibody complexes were detected with ^{125}I-labeled protein A as described. Sizes are shown in kDa.

SSB-1 or a gene regulating the expression of SSB-1. In the light of the existence of the multiple species of SSBs in higher eukaryotes and their suggested immunological relationships, it is worth pointing out that an extra protein band (about 55 kDa) is observed in both samples shown in Fig. 2, even though affinity-purified antibodies were used in the experiment.

Immunoblotting was used to estimate the number of molecules of the SSB-1 protein in cells (data not shown). In strains containing a single gene, there are about 20,000 molecules of SSB-1 protein per haploid cell. Thus, the overproducing strain contains up to 60,000 molecules of 0.4% of total soluble protein. Overproduction to this extent had no effect on cell growth.

Localization of the SSB-1 Gene Within the Insert. Overproduction as measured by immunoblotting of strains carrying an intact *SSB1* gene in YEp24 also provides a method to map the location of the SSB-1 coding sequence within the 6.5-kb insert in pSB1-4. A series of plasmids carrying overlapping deletions was constructed by digestion with various restriction enzymes (see Fig. 1). Plasmids containing the deletions were introduced into yeast cells, and transformants were examined for their ability to overproduce SSB-1. The gene, which is estimated to be about 1.2 kb in length, was localized between two *Bgl* II sites (about 1.95 kb, Fig. 1). In order to further define the limits of the gene, a 1.5-kb DNA fragment was inserted at the *Bam*HI site within this 1.95-kb *Bgl* II fragment. Disruption of the *Bgl* II fragment did not interfere with the overproduction of the native protein, suggesting that the entire *SSB1* gene, including all essential 3′ and 5′ flanking regions is within the 1.84-kb *Bgl* II–*Bam*HI fragment (Fig. 1). A detailed map of the *Bgl* II–*Bam*HI fragments is shown in Fig. 1*C*.

If expression of foreign DNA inserted into λgt11 occurs as a fusion protein with β-galactosidase, the coding sequence for the foreign gene should lie directly downstream from the *lacZ* sequences. Surprisingly, however, restriction mapping of the two λ clones described above indicated that the *SSB1* gene was not fused to either *lacZ* or λ sequences, but lay entirely within the yeast insert. It is not clear what signal allows expression of this gene.

Production of Yeast SSB-1 Protein in *E. coli*. In order to verify that the *Bgl* II fragment contains the *SSB1* structural gene, the gene was inserted into an *E. coli* expression vector, and production of yeast SSB-1 in *E. coli* was monitored by protein blotting. We have used the T7 RNA polymerase/promoter system to produce SSB-1 protein in *E. coli* (14). A protein immunologically related to SSB-1 with a mobility of 45 kDa is produced in *E. coli* (Fig. 3, lane a′). The appearance of this protein occurs only in strains carrying plasmids with the *SSB1* gene and only after induction of T7 RNA polymerase. Only plasmid pT7-a gives rise to the SSB-1-related protein (Fig. 3). The orientation of the gene in pT7-a is consistent with the direction of transcription of the *SSB1* gene as determined by RNA blots of yeast extracts using RNA probes synthesized *in vitro* by T7 RNA polymerase and pT7-a or pT7-b template (data not shown). The direction of transcription of the *SSB1* gene is indicated in Fig. 1. Interestingly, the affinity-purified SSB-1 antibodies react with a 26-kDa *E. coli* protein and not with purified *E. coli* SSB (19 kDa) (12).

Gene Disruption. Gene disruption was carried out by homologous recombination of a cloned internal fragment of the *SSB1* gene with the genomic DNA (20). From the size of the SSB-1 protein and the deduced size of the gene (1.2 kb), the 615-base-pair *Eco*RI–*Hpa* I fragment shown in Fig. 1*C* should lie entirely within the coding sequence. The *Eco*RI–*Hpa* I fragment was isolated, and a *Xho* I linker was inserted into the *Hae* III site of the fragment. Plasmid pGX6 was then constructed by insertion of the modified *Eco*RI–*Hpa* I *SSB1* fragment into plasmid YIp5 between the *Eco*RI and *Bam*HI sites. To increase the transformation frequency and target the transforming DNA to the endogenous *SSB1* gene, we digested pGX6 with *Xho* I enzyme to form a linear plasmid prior to transformation into the diploid yeast strains JLC3 or SEY-2101/SEY2102. Selection for uracil prototrophy (URA$^+$) yielded 10–20 transformants per μg of linear plasmid. Integration at the *SSB1* locus was confirmed by the Southern blotting as shown in Fig. 4. The 1.95-kb *Bgl* II chromosomal fragment contains one intact copy of the *SSB1* gene, and the 7.7-kb fragment contains the plasmid pGX6 integrated into the other chromosomal *SSB1* gene copy (1.95 kb plus 5.8 kb of plasmid DNA). There is a 2.9-kb band that hybridizes less strongly but detectably with the *SSB1* probe. The *Bgl* II restriction map of the *SSB1* region indicates that no partial digestion product containing the *SSB1* gene could be produced with a size of 2.9 kb. Whether this represents a related gene has not yet been established.

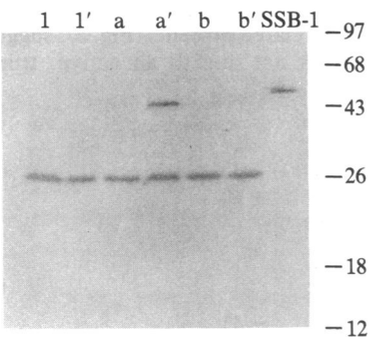

FIG. 3. Expression of yeast *SSB1* in *E. coli*. *E. coli* strain K-38 was transformed by the plasmids pT7-1 (vector), pT7-a, or pT7-b (vectors containing the *SSB1 Bgl* II fragment in two orientations). Heat induction to express the inserted gene is described by Tabor and Richardson (14). The figure shows an autoradiogram of a protein blot using affinity-purified yeast SSB-1 antibodies and extracts of *E. coli* carrying the various plasmids: pT7-1 without heat induction (lane 1); pT7-1 with heat induction (lane 1′); pT7-a without heat induction (lane a); pT7-a with heat induction (lane a′); pT7-b without heat induction (lane b); and pT7-b with heat induction (lane b′). Purified SSB-1 protein (0.3 μg) shown on the right-most lane. The standard protein markers are phosphorylase b (97 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), α-chymotrypsinogen (25.7 kDa), β-lactoglobulin (18.4 kDa), and cytochrome *c* (12.3 kDa).

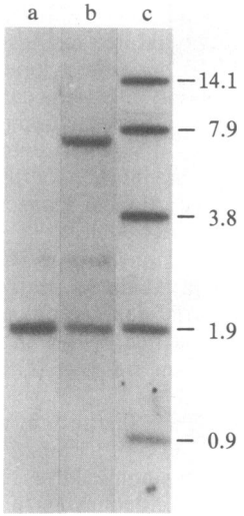

FIG. 4. Southern blotting of normal diploid cells and of diploid cells carrying one disrupted copy of the *SSB1* gene. Total DNA (4 μg) from each strain was cleaved with restriction enzyme *Bgl* II. After electrophoresis on a 1.0% agarose gel, DNA fragments were transferred to nitrocellulose filter paper and hybridized with a ^{32}P-labeled *Eco*RI–*Hpa* I fragment, which should be entirely within the *SSB1* gene. Lanes: a, parental diploid; b, diploid containing the disrupted gene: c, molecular weight markers produced by digestion of the plasmid pSB1-4, which contains the *SSB1* gene, with various restriction enzymes. The *SSB1* gene is located on each of the fragments, which were generated as follows: pSB1-4/*Bam*HI (14.1 kb), pSB1-4/*Hin*dIII (7.9 kb), pSB1-4/*Kpn* I (3.8 kb), pSB1-4/*Bgl* II (1.9 kb), and pGX6/*Eco*RI + *Sal* I (0.9 kb).

Transformants carrying the disrupted gene were sporulated, and tetrad analysis was carried out. A total of 20 tetrads was dissected by micromanipulation, and 18 of 20 transformants gave four viable spores, indicating that SSB-1 is not essential for the growth of cells. The growth rates of all germinated spores were identical. Further analysis of the spores of a single tetrad by Southern blotting showed that, as expected, the two URA$^+$ spores contained the disrupted gene and the two URA$^-$ spores contained the intact gene. All other markers also segregated 2$^+$:2$^-$. Strains derived from spores containing a disrupted gene produce no SSB-1 and no novel immunologically related proteins (Fig. 5), suggesting that the ability to grow is not due to an active, truncated SSB-1 protein. Importantly, the absence of SSB-1 from strains carrying the disrupted gene confirms that it is the *SSB1* gene that has been cloned. The blot in Fig. 5 also shows two related proteins of 55 kDa and 75 kDa that are present in all yeast strains. Their mobility is unchanged by the gene disruption. In summary, yeast cells contain a single copy of the *SSB1* gene and perhaps a second copy of a related gene. Disruption of the *SSB1* gene is not lethal to the cells.

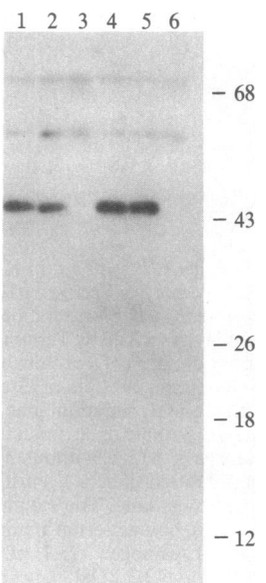

FIG. 5. Protein blotting of diploid or haploid cells containing the normal or disrupted *SSB1* gene. Protein (10 μg) from an extract of each strain was separated by gel electrophoresis and blotted as described. Size standards (shown in kDa) are described in the legend to Fig. 3. Lanes: 1, parental diploid; 2, diploid containing one disrupted copy of the *SSB1* gene; 3–6, protein from four spores of a single tetrad, after the diploid in lane 2 was sporulated.

DISCUSSION

A 1.8-kb chromosomal DNA fragment for *S. cerevisiae* that carries the structural gene encoding SSB-1, a yeast single-stranded nucleic acid binding protein (12, 21), has been isolated. The identity of the gene was proved by the following criteria: (*i*) SSB-1 is overproduced 3-fold in a yeast strain that carries a high-copy-number plasmid vector, YEp24, containing the *SSB1* gene; (*ii*) an immunologically related protein has been produced in *E. coli* by expressing the yeast *SSB1* gene there; and (*iii*) disruption of the gene results in loss of the SSB-1 protein as judged by protein blots. The *SSB1* gene has been used to construct a mutant deficient in this protein.

The λgt11 vector that was used to isolate the *SSB1* gene is designed to express part of a foreign protein fused to the β-galactosidase encoded on the vector. When a restriction map of the intact *SSB1* gene isolated from a plasmid library containing genomic DNA was compared to the λgt11 clones, however, the *SSB1* gene was not found to be fused to the *lacZ* region of the λgt11 vector. Instead the *SSB1* gene was located about 1.5 kb away from *lacZ*, with the direction of transcription of *SSB1* opposite to that of *lacZ*. Previously, Goto and Wang (22) found that the *TOP2* gene cloned in λgt11 was oriented so that the direction of transcription was opposite to that of β-galactosidase. Thus, expression of foreign DNA in λgt11 is not always dependent on expression of β-galactosidase and can be independent of the orientation of the gene.

Biochemical assay of the SSB-1 protein is difficult because the only enzymatic assay lies in its stimulation of yeast DNA polymerase I. However, the SSB-1 protein can be used in the stimulation assay only if it is first purified near to a homogenous form (12). Since it would be extremely tedious to use this method to verify that the clones contained the *SSB1* gene, we used immunoblotting to quantitate levels of SSB-1 and overproduction of proteins for identification and mapping of the gene. In order to rule out the possibility that the 1.8-kb DNA fragment encodes a regulatory protein that stimulates the synthesis or inhibits the turnover of the SSB-1 protein, even though the gene was isolated from an expression library, we demonstrated that cloning of *SSB1* gene in *E. coli* allows expression of an immunologically related protein in *E. coli*.

One of the purposes in isolating the *SSB1* gene was to overproduce the protein to obtain large quantities for biochemical characterization. The *E. coli* system used is the phage T7 expression vector system of Tabor and Richardson (14). Expression occurs only after heat induction in this system, which is inconvenient on a large scale. Furthermore, in some cases, the accumulation of SSB-1 caused lysis of *E. coli* (unpublished results). Thus, optimal conditions for the large-scale production of yeast SSB-1 protein in this system have not yet been established. On the other hand, the overproduction of SSB-1 protein in yeast cells, though only 3-fold in magnitude, is reproducible, and the protein is stable. While the protein is overproduced only 3-fold, we observed 10 times the normal level of the mRNA. Previously, genes *CDC8* (temperature-sensitive cell-cycle arrest) (23) and that for the 11-kDa subunit of the ubiquinol cytochrome reductase (23) cloned into a high-copy-number 2-μm origin-carrying vector produced similar quantities of mRNA (15- to 30-fold) and protein (6- to 8-fold). Quantitative analysis of both

mRNA and protein levels suggests that both translational controls and elevated turnover of excess protein contribute to a partial compensation for the effects of increased gene dosage in transformed cells (24). The level of SSB-1 also may somehow be regulated, since the amounts of mRNA and protein are not identical.

A plasmid containing an internal *SSB1* gene fragment was integrated into the chromosomal *SSB1* locus to disrupt the gene. Haploid cells containing the disrupted gene are viable. It is difficult to understand why yeast cells carry such an abundant (20,000 copies per cell) yet nonessential protein. Furthermore, SSBs are thought to be involved in DNA replication and RNA processing, which should be essential for cell viability (see the Introduction). One possible explanation of our observation is that the disrupted gene product is still functional. A truncated SSB has been shown to be functional in the adenovirus system, where the 59-kDa DNA binding protein was digested by chymotrypsin to produce a truncated 34-kDa carboxyl-terminal polypeptide, which could still complement adenovirus DNA replication in nuclear extracts of cells infected with a mutant deficient in the 59-kDa DNA binding protein (25). However, it is doubtful that such an explanation applies in our case because we were not able to detect any SSB-1 or novel immunologically related protein on protein blots of strains containing the disrupted gene. Another possibility is that a second gene product may substitute for SSB-1 *in vivo*. This suggestion is particularly interesting because affinity-purified antibodies to SSB-1 crossreact with two larger proteins of 55 kDa and 75 kDa on protein blots (Fig. 5 and ref. 12). It is unlikely that these proteins are encoded by the *SSB1* gene, since they are not affected by the gene disruption. DNA sequencing of the *SSB1* gene will be required to determine the size of the primary translation product, however. Therefore, it is possible that there is a second gene encoding a related protein. Several other yeast genes, such as the *RAS* genes (26), histones H2A (27) and H2B (28), and the ribosomal protein p51 (29) are encoded by two unlinked genes. Disruption of only one member of the gene pair causes no phenotypic change, whereas disruption of both genes has severe consequences on cell viability. A number of other genes that encode abundant proteins are known to be repeated in yeast (see ref. 30), such as genes for glyceraldehyde-3-phosphate dehydrogenase (three copies of the gene) and acid phosphatase (two copies), the *MAL* gene, and the *SUC* gene (several copies). Also, there are two cytochromes *c* in yeast, iso-1-cytochrome *c* (95%) and iso-2-cytochrome *c* (5%), encoded by related but not identical genes (31). The genes of yeast SSBs may be related in the same manner as those of cytochrome *c*. In summary, the results presented here suggest the possibility of a gene family encoding related SSBs in yeast. In higher eukaryotes, as pointed out in the Introduction, the various classes of SSBs crossreact immunologically suggesting that higher cell eukaryotic SSBs are also encoded by a gene family. Further genetic and biochemical analysis of yeast SSBs will reveal the biological significance of these findings.

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