The Single-Stranded DNA-Binding Protein of Escherichia coli

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### INTRODUCTION AND SCOPE OF REVIEW

The single-stranded DNA-binding protein (SSB) of *Escherichia coli* plays a central role in the cell by participating in the “three R’s” of DNA metabolism: replication, repair, and recombination. Moreover, as this protein binds tightly and cooperatively to DNA, it has become a prototypic protein in the study of protein-nucleic acid interactions. It is our intention to provide for the first time a comprehensive summary involving all aspects of the *E. coli* SSB in a single review. We shall examine the structure of the protein, its interaction with other proteins and DNA, the *ssb* gene, the physiological effects of *ssb* mutations, and mechanistic insights into the role of SSB in replication, repair, and recombination. Most earlier reviews have focused on the interactions of SSB with DNA, usually in comparison with other DNA-binding proteins, or have included small sections on SSB in conjunction with a broader topic such as repair or recombination. Earlier reviews of SSB include references 70, 76, 91, 133, 188, 220, and 419. The literature in the current review has been surveyed through December 1989.

### HISTORICAL PERSPECTIVE

The study of DNA-binding proteins, in general, dates from the development of DNA-cellulose affinity chromatography in the laboratory of Bruce Alberts in the late 1960s (4, 7), which led to the discovery of gene 32 protein of bacteriophage T4 (7). Some of the interesting features of this phage protein noted at that time included very tight binding to DNA-cellulose, requiring 2 M NaCl for elution from the column. This protein bound tightly, selectively, and cooperatively to single-stranded DNA (ssDNA) (7, 103). Shortly thereafter, Alberts, in collaboration with investigators in Gefter’s laboratory, discovered an analogous protein in uninfected *E. coli* cells (356). They named this protein the *E. coli* DNA-unwinding protein because of its ability to unwind (i.e., destabilize) a DNA double helix (356). Over the years this protein has been known by a variety of names including DNA-unwinding protein (270, 356), DNA-binding protein (234, 272, 273), DNA-binding protein I (131), DNA-melting protein, DNA-extending protein, and helix destabilizing protein (5). With the discovery of the gene for this protein (257), we coined the term single-strand-binding protein (SSB), the product of the *ssb* gene. This name was chosen to reflect the fact that it could also bind RNA (273, 327, 404), albeit with far less affinity than DNA. However, since the functions of this protein are carried out while bound to ssDNA, many authors, ourselves included, have referred to this protein as the single-stranded DNA-binding protein. Both of these names continue to be used.

Early research specifically focused on SSB was carried out primarily in the laboratories of Gefter (270–273, 356) and Kornberg (404) and dealt with purification and general characterization of SSB. The identification in 1979 of the *ssb* gene and *ssb* mutations (136, 257, 259) led to an explosion of information in the subsequent decade, with several laboratories examining one or more aspects of SSB structure or function, to be summarized below.

### PHYSICAL PROPERTIES OF SSB

#### Purification

*E. coli* SSB is an abundant and stable protein which can be purified quite easily and in very large quantities. As the *ssb* gene has been cloned on λ phage and on a variety of plasmids (see the section on cloning of the *ssb* gene), up to 300-fold overproduction has been achieved (222, 422). Early purification methods included the use of DNA affinity columns, as a high degree of purification could be achieved in a single...
TABLE 1. General properties of E. coli SSB

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M'd wt of monomer by SDS-polyacrylamide gel electrophoresis</td>
<td>18,500*</td>
<td>404</td>
</tr>
<tr>
<td>Amino acid residues</td>
<td>177</td>
<td>333</td>
</tr>
<tr>
<td>Stokes radius (nm)</td>
<td>3.8-3.9</td>
<td>404, 423</td>
</tr>
<tr>
<td>Frictional coefficient</td>
<td>1.36-1.42</td>
<td>404, 423</td>
</tr>
<tr>
<td>Sedimentation value, (S20, w)</td>
<td>4.65*</td>
<td>423</td>
</tr>
<tr>
<td>Diffusion coefficient, (D20, w) (cm/s)</td>
<td>5.6 x 10^-7</td>
<td>404</td>
</tr>
<tr>
<td>pH</td>
<td>6.0</td>
<td>404, 423</td>
</tr>
<tr>
<td>λ (280 nm)</td>
<td>28,500*</td>
<td>43</td>
</tr>
<tr>
<td>A280/A350</td>
<td>1.8</td>
<td>404</td>
</tr>
<tr>
<td>Copies per cell</td>
<td>1,000-2,000</td>
<td>43</td>
</tr>
</tbody>
</table>

* Literature values vary from 18,000 to 22,000 (270, 326, 340, 356, 404).
* Literature values vary from 4.3 to 5.3 (194, 404, 427).
* Value reported is for the monomer. Other reported values range from 23,700 to 33,000 (43, 44, 149, 188, 191, 220, 223, 326, 350, 422, 423). See references 43 and 220 for a discussion.

The use of plasmids that place ssb under control of the λ P1 promoter results in an overexpression of ssb to the point that ca. 10% of the soluble protein is SSB. Yields of ca. 3 mg of SSB per g of cells can be collected in 8 h or less (222). Purified SSB is also available commercially from several sources.

**General Properties**

Some physical properties of SSB are summarized in Table 1. Although earlier estimates of monomeric molecular weight, as determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, ranged from 18,500 to 22,000 (191, 270, 356, 404), DNA sequence analyses place it at 18,843 (72, 333). In solution the protein is a stable homotetramer (149, 294, 404, 422, 423), and there is no evidence for distinct higher-order forms. SSB aggregates at protein concentrations above ca. 1 mg/ml (191, 404; R. R. Meyer and F. W. Perrino, unpublished observations) and at low ionic strength (66, 174, 191); such aggregates are biologically inactive. Even at lower protein concentrations, aggregation occurs over time upon storage at 4°C. These aggregates can be demonstrated by electrophoresis under non-denaturing conditions (F. W. Perrino and R. R. Meyer, unpublished observations). E. coli SSB is an extremely stable protein and is resistant to heat denaturation with only 10% loss of activity after boiling for 8 min (404). We have taken samples of SSB, stored at 4°C for more than 5 years, heated them in a boiling-water bath for 1 min, and found dissociation of the aggregates with restoration of >95% of the original biological activity as measured in an in vitro replication assay (R. R. Meyer, unpublished data). Fluorescence measurements have confirmed that SSB undergoes a conformational change between 40 and 70°C and that binding to poly(dT) or phage fd DNA protects it from denaturation (191). The protein is stable to alkaline denaturation, as exposure to pH 11 followed by neutralization did not alter its DNA-binding properties (30). It is also resistant to treatment with 4 M guanidine hydrochloride (340). We have observed some proteolytic degradation of SSB on long-term storage (more than 6 months), although this does not have much effect on biological activity (Meyer, unpublished). Others have reported apparent autoproteolytic activity during long-term storage at 4°C at high (>7 mg/ml) protein concentrations (43, 293). Both groups report the appearance of distinct-sized fragments of 13,800 and 10,500 daltons (Da) in one case (158) and 14,500 Da in the other (293). It is likely that the larger of the two fragments reported by each group are identical. At concentrations below 2 mg/ml or in buffers containing glycerol, such proteolysis was not observed (158).

Williams et al. (422) have examined the stability of SSB tetramers by high-pressure liquid chromatography gel filtration as a function of protein concentration and temperature. At 25°C there was no evidence for dissociation into monomers at the lowest concentration tested (ca. 0.04 μM or ca. 3 μg/ml), although at 45°C dissociation of tetramers was observed below 0.5 μM (ca. 37.5 μg/ml). These results have been confirmed by Lohman and Bujalowski (220). The in vivo concentration of SSB is estimated to be 0.5 to 1.0 μM (422). Therefore, under physiological conditions of temperature and ionic strength, virtually all of the SSB should be tetrameric.

There is no evidence in the literature for a stable dimeric or trimeric form in solution. Molin et al. (273) reported that SSB may bind to oligonucleotides as a monomer or dimer, as determined by changes in sedimentation rate. This, however, could not be confirmed by others, and it is now believed that the tetrameric form binds oligonucleotides (30, 149, 327). Griffith has reported the formation of nucleosome-like structures consisting of "core particles" of an octamer of SSB subunits and 145 bases of ssDNA, separated by ca. 30 bases of unbound nucleotide pairs. It should be noted that these octamers are seen only when SSB is bound to DNA and never when the protein is free in solution (83, 151).

A frictional coefficient of ca. 1.4 (Table 1) indicates that the tetramer is slightly ellipsoidal or hydrated. The protein has been crystalized in three different laboratories under differing experimental conditions (158, 274, 293). Thus far, such crystallographic studies have not yielded much information, as the crystals appear to form only after partial proteolysis (158, 293). All three laboratories obtained some crystals that were monoclinic of space group C2 and were consistent with a tetrameric structure having D4 symmetry (293).
Amino Acid Sequence

Beyreuther et al. (38) sequenced the first 40 amino acids from the NH₂ terminus of SSB by using automated Edman degradation. The complete amino acid sequence, however, was deduced from the DNA sequence first reported by Sancar et al. (333). These authors also sequenced the first 52 NH₂-terminal amino acids and found complete agreement with the DNA sequence. A segment of the carboxy terminus and additional internal segments have been sequenced during determination of the amino acid changes in ssb mutants, and these protein sequences confirm those predicted from the DNA sequence (72, 422). A molecular weight of 18,873 was calculated for the SSB monomer. A subsequent correction to the sequence (72) indicated that glycine rather than serine is present at position 133, with a concomitant adjustment of the molecular weight to 18,843. Figure 1 shows the complete DNA and protein sequence of the 177 amino acids, obtained from GenBank and corrected as indicated (72). The N terminus is an Ala residue, indicating that cleavage of the Met initiation amino acid has occurred. The amino acid composition, determined from the DNA sequence, is given in Table 2. Amino acid analyses had been reported earlier (9, 404) but varied somewhat from that determined from the DNA sequence (333). The striking features of the amino acid composition are the relatively high content of Gly, accounting for nearly one of every seven amino acids, the lack of any Cys residues, and the single His (Table 2). The carboxy terminus (12 amino acids, residues 166 to 177) is unusual in that it is highly negatively charged (charge density of −5) and shows an extensive predicted α-helical structure (Fig. 2). It is quite similar in this respect to the gene 32 helixdestabilizing protein of phage T4 (420) (see the discussion on protein domains below). Figure 3 shows a hydrophobicity plot of the sequence. The predominance of hydrophilic domains (below the solid horizontal line at 0) is apparent. There are four major hydrophobic domains, three of which occur in the N-terminal third of the protein. The third hydrophobic domain (arrow) encompasses sequences important for DNA binding and subunit interaction (see section below). Analysis of the predicted antigenic sites revealed high Kyte index scores for coordinates 88, 52, 90, 93, and 94, respectively, in order of decreasing values (Meyer, unpublished).

Functional Domains

Secondary structures. Using circular dichroism to determine secondary structure, Anderson and Coleman (9) reported that SSB contained approximately 20% α-helix, 20% β-sheet, and 60% random coil. From the sequence

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>No. of residues</th>
<th>% Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala (A)</td>
<td>13</td>
<td>7.3</td>
</tr>
<tr>
<td>Arg (R)</td>
<td>10</td>
<td>5.6</td>
</tr>
<tr>
<td>Asn (N)</td>
<td>8</td>
<td>4.5</td>
</tr>
<tr>
<td>Asp (D)</td>
<td>8</td>
<td>4.5</td>
</tr>
<tr>
<td>Cys (C)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Glu (E)</td>
<td>10</td>
<td>5.6</td>
</tr>
<tr>
<td>Gln (G)</td>
<td>18</td>
<td>10.2</td>
</tr>
<tr>
<td>Gly (G)</td>
<td>29</td>
<td>16.4</td>
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<tr>
<td>His (H)</td>
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<tr>
<td>Ile (I)</td>
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<td>Leu (L)</td>
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<td>4.5</td>
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<td>Lys (K)</td>
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<tr>
<td>Met (M)</td>
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<td>Phe (F)</td>
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<td>2.3</td>
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<tr>
<td>Pro (P)</td>
<td>12</td>
<td>6.8</td>
</tr>
<tr>
<td>Ser (S)</td>
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<td>5.6</td>
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<td>Thr (T)</td>
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<td>4</td>
<td>2.3</td>
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<tr>
<td>Tyr (Y)</td>
<td>4</td>
<td>2.3</td>
</tr>
<tr>
<td>Val (V)</td>
<td>13</td>
<td>7.3</td>
</tr>
</tbody>
</table>

* Determined from the DNA sequence.
charged amino acids. The numbers indicate residues at conformational boundaries. The known ssb mutational sites are indicated by arrows. Modified and reprinted from the Proceedings of the National Academy of Sciences (333) with permission of the publisher.

data and by using the Chou and Fasman method (79) to predict secondary structure, values of 22% α-helix, 19% β-pleated sheet, and 59% random coil were obtained (333), which is in good agreement with the earlier estimate (9). Figure 2 shows the predicted secondary structure of SSB (333). Most of the highly ordered structure is found in the NH2 terminus from residues 1 to 105. This region also contains most of the charged amino acids, especially the positively charged amino acids (14 of 16 residues). This is followed by a long stretch of random-coil sequence (residues 106 to 165), which is also unusual in lacking any charged amino acids and in which ca. 80% of the residues are glycine, proline, glutamine, and asparagine. The COOH terminus (residues 166 to 177) is characterized by a short region of α-helix and several acidic amino acids.

Subunit interaction domain. Studies by Williams et al. (422) on the SSB-1 mutant protein (His-55→Tyr [Fig. 2; Table 2]) have suggested that the subunit interaction domain of SSB includes the region of the protein which is also involved in DNA binding (see section). The ssb-1 mutation results in temperature-sensitive DNA replication (257, 258). Williams et al. (422) examined the monomer-tetramer equilibrium and found that isolated SSB-1 readily dissociates into monomers even at temperatures that are permissive (<42.5°C) for ssb-1 cells. The in vivo SSB or SSB-1 concentration has been calculated to be 0.5 to 1.0 μM (422). At these levels at 25°C, a significant proportion of SSB-1 should be in the monomeric form. At the nonpermissive temperature (45°C), similar dissociation was observed for SSB-1. However, fluorescence quenching experiments (422) indicated that at these physiological protein concentra-

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Two other observations are relevant in this regard. Mutant SSB-1 is only temporarily inactivated by heating to 42°C (258). This is illustrated in Fig. 4, which shows that in vitro DNA replication, using a G4 phage complementary-strand-to-replicative-form (SS→RF) assay and purified SSB-1 protein, stops within 1 min of temperature shift to 42°C and resumes within 1 min of shift back to 20°C (258). Therefore, the mutant SSB-1 monomers were not irreversibly denatured. Second, as indicated earlier, SSB is a heat-stable protein (404). We have shown that SSB-1 can be boiled briefly and still retain biological activity (258). Consequently, the mutant protein is heat stable yet functionally temperature sensitive! Surprisingly, boiling increases the efficiency of SSB-1 in in vitro assays, suggesting that a conformational change has been induced in the protein that provides more stability to SSB-1 tetramers (258; Meyer, unpublished). It should be noted that although wild-type SSB and temperature-sensitive mutants SSB-1 and SSB-113 are heat stable and retain full biological activity, the binding properties are changed by the heating.

In view of the fact that truncated SSBc and SSBt (proteolytically produced SSB fragments; 423) can form tetramers, it is unlikely that the carboxy terminus is required for this process. Since nothing is known of the relative affinities of the truncated forms for tetramer formation, a modulating effect of the carboxy terminus cannot be ruled out. At present we know that His-55 is involved in subunit interaction, but what other amino acid residue(s) is involved remains to be determined.

**DNA-binding domain.** The functional domains of SSB have been probed in a variety of ways. Using photochemical cross-linking of SSB bound to oligo(dT)n, Merrill et al. (254) have identified Phe-60 as the site of cross-linking (Fig. 2 and 3). The role of Phe-60 in DNA binding was later confirmed by site-specific mutagenesis in which an alanine was introduced at position 60 (65; a list of ssb mutations is given in Table 3). Fluorescence titration data showed that substitution of alanine at this position reduces the affinity of the mutant protein for poly(dT) by 3 orders of magnitude com-

**Amino Acid Position**

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TABLE 3. E. coli SSB mutations

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Mutational site</th>
<th>Amino acid change</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ssb-1</td>
<td>55</td>
<td>His→Tyr</td>
<td>257, 422</td>
</tr>
<tr>
<td>ssb-2</td>
<td>?</td>
<td>Unknown</td>
<td>24</td>
</tr>
<tr>
<td>ssb-3</td>
<td>15</td>
<td>Gly→Asp</td>
<td>339a; Tessman, personal communication</td>
</tr>
<tr>
<td>ssb-113</td>
<td>176</td>
<td>Pro→Ser</td>
<td>72, 145, 259</td>
</tr>
<tr>
<td>ssb-114</td>
<td>176*</td>
<td>Pro→Ser</td>
<td>76, 164</td>
</tr>
<tr>
<td>ssbW40F</td>
<td>40</td>
<td>Try→Phe</td>
<td>172</td>
</tr>
<tr>
<td>ssbW54F</td>
<td>54</td>
<td>Try→Phe</td>
<td>172</td>
</tr>
<tr>
<td>ssbW88F</td>
<td>88</td>
<td>Try→Phe</td>
<td>172</td>
</tr>
<tr>
<td>ssbF60A*</td>
<td>60</td>
<td>Phe→Ala</td>
<td>65</td>
</tr>
<tr>
<td>ssbH55L*</td>
<td>55</td>
<td>His→Leu</td>
<td>65</td>
</tr>
<tr>
<td>Δssb</td>
<td></td>
<td>Gene deleted</td>
<td>299</td>
</tr>
</tbody>
</table>

\* The ssb-114 mutation has been reported to have the same amino acid change as the ssb-113 mutation (76). However, Johnson (164) reported differing physiological effects between ssb-113 and ssb-114 mutations, which suggests that the ssb-114 mutation may contain an additional amino acid change (see text).

\* The mutational designation used follows that of Khamis et al. (172) for mutations produced by site-directed mutagenesis.

Upon binding to DNA, SSB displays ca. 70% quenching of fluorescence observed at 345 nm when excited with UV light. This was first observed by Molineux et al. (273), who suggested that such quenching was due to interaction of tryptophan(s) in binding the nucleic acid. The degree of fluorescence quenching depends upon the number of binding sites on the tetramer that are actually in contact with the DNA, and at full saturation the quenching is on the order of 80 to 89% (61, 191, 220). As tyrosine residues also result in fluorescence emission and there are four tyrosine residues in SSB (404) located at positions 22, 40, 48, and 97 (333) (Fig. 1 and 2), these early experiments (273) could not rule out participation of tyrosine(s) in DNA binding. Later analyses, using differential fluorescence spectra, were able to show that tyrosines did not contribute to the fluorescence quenching (30). Moreover, chemical modification experiments (9) indicated that none of the tyrosines of SSB are exposed for nitration, lending further support to a lack of involvement of tyrosine(s) in DNA binding. Treatments resulting in modification of arginine, cysteine, or tyrosine residues had no effect on binding of SSB to DNA, whereas modification of either lysine residues (with acetic anhydride) or tryptophan residues (with N-bromosuccinimide) led to complete loss of binding activity (30). The most definitive work has come from Maki’s laboratory in a series of publications beginning in 1984 (66, 68, 171–174, 428), in which fluorescence quenching was augmented by optically detected triplet-state magnetic resonance spectroscopy. There are four tryptophan residues in SSB located at positions 40, 54, 88, and 135 (Table 2; Fig. 1 and 2). Initial studies suggested that one or two of these tryptophans were involved in binding (68, 171). Two important experimental approaches have led to the specific identification of the tryptophan residues involved. The first was a comparative study of SSBs specified by naturally occurring conjugative plasmids of enteric bacteria (142, 177). Many of these SSBs have been sequenced and shown to have extensive homology to the E. coli chromosome-encoded SSB, especially in the NH2-terminal region responsible for DNA binding (73). Tryptophan 135, which is found in a polar environment and at a distance from the conserved NH2 terminus, is absent in SSBs specified by plasmids pIP71a, R64, F, and pIP231a (64, 174). As fluorescence quenching and optically detected triplet-state magnetic resonance spectroscopy data were qualitatively the same for such SSBs as they were for the E. coli chromosomal SSB (64, 174), this eliminated Try-135 as playing a role in DNA binding. This conclusion is strengthened by the observation that the SSBT fragment produced by limited proteolysis lacks a tryptophan at site 135, yet has no reduction in affinity for single-stranded nucleic acids (423). Of the three remaining tryptophans, only two were implicated in DNA binding (174). The second approach involved site-specific mutagenesis to produce several individual mutations in which each tryptophan was changed to phenylalanine. These SSBs were purified and used to determine the ability of these mutant proteins to bind single-stranded polynucleotide and to determine the spectral changes that occurred upon binding (Table 3) (65, 172, 173, 428). These studies showed that Try-88 had no effect, but both Try-40 and Try-54 were involved and responsible for the fluorescence quenching observed upon binding of SSB to nucleic acids (172).

Site-specific mutagenesis has also been used to produce amino acid changes at sites 60 (Phe→Ala) and 55 (His→Leu) (Table 2) (65). As indicated above, Phe-60 is the site that can be photochemically cross-linked to nucleotides (254). Sub-
stution of Ala for Phe at site 60 resulted in a decrease in binding affinity of 3 orders of magnitude. Substitution for His at site 55 was estimated to reduce binding affinity by 6,000-fold (65). The His-55 site is also the position of the ssb-1 mutation (His-55→Tyr [Table 3]), and this substitution may disrupt the α-helix. SSB-1 has ca. 2,000-fold reduced affinity for DNA. The observation that SSB-1 does not protect DNA from nuclease attack as well as SSB does (258) lends further support to the importance of this domain in DNA binding.

From these studies, the binding of single-stranded DNA has been shown to involve a region between Try-54 and Phe-60 (Try-54→His-55→Arg-56→Val-57→Val-58→Leu-59→Phe-60→). This region is hydrophobic (3) and probably α-helical (Fig. 2), with Try-54 and Phe-60 separated by approximately two helical turns (172). Space-filling models show that there is a cavity between these two amino acids (Try-54 and Phe-60) which could accommodate one or two stacked pyrimidine rings (65). It should be noted, however, that not all authors agree with this interpretation, and at least one group (300) has argued that this binding domain (from residues 40 to 65) is more likely to assume a β-sheet configuration. Models involving stacking interactions through hydrophobic interactions between aromatic amino acids and nucleotide bases have been reviewed by Hélène and Maurizot (155) and are thought to be an important mechanism in the binding of such proteins as SSB to ssDNA. Electrostatic interactions of basic residues with the phosphates of DNA are also thought to play a role in DNA binding (155), and, as indicated above, this involves a lysine residue(s) but not arginine residues (30). Although three of the four Lys residues of SSB lie in the region (residues 43, 49, and 62), the important ones have yet to be determined. It is also interesting that the addition of 40 amino acids to the NH2 terminus by using a fusion protein-cloning vector system (341) had no apparent effect on DNA binding or on subunit interaction. Recently McHenry has been able to observe the kinetics of dissociation of SSB from ssDNA by monitoring the recovery of fluorescence emitted by free SSB (C. S. McHenry, personal communication).

Protein-protein interaction domains. Very few direct experimental data are available on the domains which interact with other proteins. However, it has been speculated that the COOH terminus may provide an interaction domain (71, 72, 149). This is based on several observations. The ssb-113 mutation lies in the penultimate amino acid (Pro-176→Ser [Table 3]), resulting in severe physiological effects (3), resulting in the section on the ssb mutations). However, SSB-113 is not impaired in DNA binding and, indeed, appears to bind polynucleotides just as well as wild-type SSB does (72). SSB-113 is slightly more effective than SSB in helix-stabilizing ability (72). Therefore, the defect in SSB-113 does not lie in its interaction with ssDNA. Chase et al. (71) have isolated a monoclonal antibody to SSB which does not react with SSB-113 in competitive radioimmunoassays. This indicates that the epitope recognized includes the COOH terminus and, furthermore, that this domain probably has an altered tertiary structure. Greipel et al. (149), using nuclear magnetic resonance spectroscopy, report that phenylalanines in the COOH-terminal domain, at positions 147, 171, and 177, are highly flexible and remain so upon DNA binding while the rest of the SSB is fairly immobile. This observation, coupled with those discussed in the section of DNA-binding domain, that the proteolytic fragment SSBC lacking the carboxy terminus is more globular and binds polynucleotides even tighter than the intact protein, support this hypothesis. Unfortunately, physiological studies with in vitro DNA replication systems, which could test this directly, have yet to be carried out. If the carboxy terminus interacts with DNA polymerase III holoenzyme during elongation and protein n' during priming (16, 352), and if SSB is displaced by these proteins (15), SSBC should not support either priming or elongation in vitro. Furthermore, the monoclonal antibody of Chase et al. (71) may also be useful in addressing this question, as it should inhibit in vitro DNA replication with SSB but be ineffective in reactions with SSB-113 when carried out at 30°C, a temperature at which SSB-113 is active (259). As noted earlier, Egner et al. (107) have shown that the SSBC fragment will support in vitro DNA recombination reactions. The latter studies would suggest either than the COOH terminus is not important for recombination and that other domains interact with RecA protein or that no direct interaction between RecA protein and SSB is necessary.

Recent observations on the properties of the ssb-3 mutation (339a) provide some interesting information, as this mutation lies at residue 15 in the N terminus (Gly-15→Asp). This mutation results in extremely severe effects on DNA repair, with only a small effect on DNA replication (R. R. Meyer and P. S. Laine, unpublished data). This argues that the N-terminal domain is important in recombinational repair, perhaps through interactions with RecA protein. This residue lies at the juncture between the first hydrophobic domain and a hydrophilic domain (Fig. 3). It would also suggest that hydrophobic interactions may be important for recombination.

Sequence Homology with Other ssDNA-Binding Proteins

Prasad and Chiu (300) have made a careful study of the primary sequences of several ssDNA-binding proteins with strong, cooperative, nonspecific binding. These included gene 5 protein from phage M13; PIKE protein from phage IKe; gene 32 protein from phage T4; and RecA, SSB, and SSB-F from E. coli. These proteins, with the exception of the E. coli chromosomal and F-factor SSBs, lack any strong overall homology. However, there are some striking similarities in the domain putatively involved in DNA binding, with aromatic and charged residues seemingly conserved. A similar sequence comparison between the T7 DNA-binding protein and SSB, made by Argos et al. (22), indicated extensive homology between these two proteins, with 24% of the amino acids being identical and another 47% representing conserved charges. This is not surprising, since the T7 polymerase can use the E. coli SSB in replication.

The most striking homologies are apparent from comparisons of the ssb sequence with that of SSBs from conjugative plasmids carried in E. coli, as deduced from DNA sequence data. The first evidence for such plasmid-encoded DNA-binding proteins was provided by observations that ssb-1 strains carrying the fertility factor F'' were partially suppressed for temperature-sensitive growth and completely suppressed if the region of the F'' responsible for suppression was cloned onto a high-copy-number plasmid (177). This plasmid-encoded gene was originally designated ssf, and the protein was designated SSB-F (142, 177). Chase et al. (73) sequenced the ssf gene and found considerable homology to ssb. In the NH2-terminal region of SSB, responsible for subunit interaction and DNA binding, fully 87 of the first 115 amino acids are identical with those of SSB-F. The DNA-binding domains are remarkable similar in the two binding proteins, with a predicted α-helical structure. The
onl other area of conserved sequence lies in the very end of the COOH terminus, where six of the last seven amino acids are the same. As discussed above (section on protein-protein interaction domains), this domain is thought to be involved in protein-protein interactions. SSB-F has 178 amino acids, one more than SSB (73), but the two proteins have a total of 106 amino acids or ca. 60% of their sequence in common. In an extensive survey of other conjugative plasmids, Golub et al. (140, 142, 143) identified 19 different plasmids carrying an ssb-like gene which could complement ssb-l cells. These genes showed extensive homology to each other and to the F’-factor ssf gene. Several of these plasmid ssb genes have been sequenced and shown to lack trypotatin at position 135 in the chromosomal SSB (64, 174), ruling out involvement of this trypotatin in DNA binding as discussed earlier (section on DNA-binding domain). The presence of ssb genes on such plasmids is of interest from an evolutionary point of view, although their exact function in the physiology of the E. coli cell still remains unknown (142).

The gene 32 protein of phage T4 has been extensively studied and often compared with E. coli SSB (for reviews, see references 76, 91, 133, 188, and 419). The gene 32 protein sequence has been published (421) and shows very little homology to E. coli SSB (333, 423). However, two features shared by these proteins are that their COOH termini are acidic and they are susceptible to proteolytic cleavage which is enhanced by binding to DNA (76). Proteolytic removal of this region affects DNA binding and increases the helix destabilizing ability of both proteins (453). The T7 DNA-binding protein shares many of these properties with SSB and gene 32 protein (423).

Barat and Mignotte (31) isolated an ssDNA-binding protein (mtSSB) from the mitochondria of Xenopus laevis. This protein is similar in some respects to E. coli SSB, including preferential and cooperative binding to ssDNA and a denatured molecular weight of ca. 15,500 (265). The protein sediments at 4S, suggesting that it is tetrameric (31), and it can stimulate DNA polymerase γ (266), the putative mitochondrial DNA-replicating enzyme. More recently, these authors have sequenced the NH₂ terminus of mtSSB and have reported two variants of the mtSSB with ca. 63% homology to the NH₂ terminus of E. coli SSB (237). These findings may have some interesting implications in terms of evolution of mitochondria (255).

INTERACTIONS OF SSB WITH OTHER PROTEINS

Direct Protein-Protein Interactions

Density gradient experiments. In the original paper on SSB by Sigal et al. (356), SSB was shown to stimulate E. coli DNA polymerase II when synthesizing DNA on long, single-stranded templates. Subsequent work by Molinex and Gefter (271, 272) reported that this polymerase forms a complex with an SSB monomer that is stable through sucrose gradient centrifugation. The precision of this technique is not great, and, as yet, there are no other confirmatory reports in the literature. Therefore, these data should be interpreted with caution. The functional significance of such an interaction with a monomer, if it exists, remains obscure but nevertheless intriguing, as SSB forms very stable tetramers in solution. The binding of polymerase II either may take place in the functional domain of SSB involved in tetramer formation or may sterically hinder tetramer formation. Alternatively, the binding of polymerase II to an SSB tetramer may promote dissociation into SSB monomers, with one remaining bound to the polymerase.

Direct protein-protein interactions of SSB have also been demonstrated by density gradient centrifugation for two other E. coli proteins, exonuclease I (272) and replication protein n (228). Exonuclease I is a 3'→5' single-strand-specific exonuclease (207), presumed to be involved in the RecF recombinational pathway (363) as well as in methyl-directed mismatch repair (201), whereas protein n is a component of the primosome complex needed for synthesis of RNA primers in lagging-strand synthesis (228). (See later sections for further details on the roles of these proteins in these processes.) As with DNA polymerase II, exonuclease I was reported to bind a monomer of SSB (272), whereas protein n, in contrast, associates with the tetrameric form (228).

The polymerase encoded by phage T7 of E. coli has also been shown by density gradient centrifugation to bind a monomer of SSB (272), although Myers and Romano (283) have failed to reproduce these results. Whereas phage T7 induces its own ssDNA-binding protein (313, 337), the T7 DNA polymerase is capable of using the E. coli SSB in in vitro DNA replication assays, as well as the T7-induced binding protein (283, 284, 337). In addition to stimulating DNA synthesis by T7 DNA polymerase and E. coli DNA polymerase II, SSB enhances the binding of these enzymes to DNA (271, 272, 283). Binding of protein n to ssDNA does not even occur in the absence of SSB (228).

SSB affinity chromatography. Direct physical interaction of SSB with several proteins was demonstrated by SSB affinity chromatography (283) with techniques originally developed by Alberts and co-workers to study phage T4 gene 32 protein-protein interactions (6, 121). After treatment with DNase I to remove DNA, cell extracts were passed over SSB-Affi-Gel 10 columns and the proteins retained were eluted with high salt or detergents. Three major proteins, with molecular masses of 25, 32, and 36 kDa, were eluted. The major 25-kDa protein was subsequently shown to be associated with the folded chromosome, perhaps as a structural component (295). Surprisingly, the binding of the 25-kDa protein was enhanced by addition of DNA polymerase III core enzyme or DNA polymerase III holoenzyme to the extracts prior to affinity chromatography. However, none of the subunits or the polymerase complex itself were retained on the SSB column. This suggests that SSB, the 25-kDa chromosomal protein, and the polymerase may interact in some sort of complex (295). The binding of the polymerase or polymerase subunit(s) may be transient or too weak to be retained on the column. In the absence of nuclease treatment of the extracts prior to chromatography, eight additional proteins ranging from 14 to 160 kDa were also bound to the SSB column. Since nucleic acids can be bound and retained on the column by the SSB matrix (295), these additional proteins may merely be interacting with the nucleic acid. Alternatively, one or more of these proteins may interact directly with SSB, but only when SSB is bound to ssDNA. Studies so far cannot distinguish between these possibilities.

SSB is capable of interacting with itself, as evidenced by the phenomenon of cooperativity when binding to a DNA lattice (see the section on DNA binding). Moreover, on SSB affinity columns, SSB is bound extremely tightly and can be eluted only under harsh conditions such as elution with a strong detergent (e.g., SDS) (295). At high protein concentrations (>1 mg/ml), SSB is known to aggregate and lose biological activity, as discussed earlier (section on general properties).

The use of SSB-affinity chromatography has been some-
what disappointing in demonstrating direct protein-protein interactions. Proteins known to physically interact with SSB, such as DNA polymerase II, exonuclease I, and protein n, could not be detected on SSB columns (295). Proteins suspected to interact with SSB, such as Rep and RecA, also were not retained when passed over such columns (Perrino and Meyer, unpublished). It is possible that such protein-protein interaction is weak or transient or occurs in solution but is disrupted if one of the proteins is attached to a solid matrix.

**Indirect Evidence for Protein-Protein Interactions**

That SSB may interact physically with other proteins is suggested by several lines of indirect evidence. The observation that Rep from certain E. coli strains can suppress both ssb-1 and ssb-113 mutations (see the section on genetic suppression), plus the fact that rep ssb double mutations are lethal, has been used to argue that a physical interaction between these two proteins may exist (380). Rolling-circle synthesis of φX174 viral plus strands has an absolute requirement for Rep, SSB, and the gene A (cistron A) protein of the virus (108, 344), which lends support to this hypothesis. However, as indicated in the preceding section, no direct interaction of SSB and Rep has yet been demonstrated. Specific cleavage of φX174 plus strands during RF→SS replication to form released viral ssDNA circles depends on SSB, suggesting a direct gene A-SSB interaction (390). Although both uvrd and ssb mutations individually confer UV sensitivity, introduction of ssb-1 or ssb-113 into a uvrd strain leads to a partial suppression of the uvrd phenotype (302). As uvrd is the gene encoding DNA helicase II (157, 241), Quiñones and Plochicki (302) have suggested that SSB and helicase II may interact physically. The helicase activity of helicase II is also known to be stimulated by SSB (245). Fassler et al. (111) have shown that rho ssb-113 double-mutation combinations are lethal, indicating a possible interaction of Rho and SSB. Further indirect evidence for protein-protein interactions has come from studies of extragenic suppressors to ssb mutations (260, 325). Such analyses have suggested an interaction of GroEL with SSB (260, 325).

Dissociation of SSB from ssDNA is very slow (131, 191, 322, 404). The observation that some proteins may promote dissociation of bound SSB argues, but does not prove, that they interact with SSB. Geider et al. (131, 430), using gel filtration of iodinated SSB complexed to ssDNA and electron microscopy, observed that phage fd gene 5 protein and E. coli HU protein were capable of displacing SSB from ssDNA, although Krauss et al. (191), using fluorescence spectroscopy, failed to confirm any competition between HU and SSB. RecA protein has also been reported to displace SSB (319).

During the SS→RF replication of phage φX174, protein n′, a DNA helicase (204), specifically recognizes a hairpin sequence of φX174 DNA (351). Upon binding SSB-coated φX174 ssDNA, some of the SSB molecules are displaced (15, 352). Upon subsequent formation of the primosome complex, more SSB is dissociated (15), and still more is dissociated during translocation of the primosome along the DNA (16). The functional interactions between SSB and other replication proteins will be discussed in more detail in the section on DNA replication.

Rothman-Denes et al. (324) have shown that early transcription of the 72-kilobase-pair double-stranded DNA (dsDNA) bacteriophage N4 genome by the phage-encoded RNA polymerase specifically requires DNA gyrase and SSB; other DNA-binding proteins are ineffective. Moreover, phage N4 cannot grow on ssb-1 host strains. In interpreting the specificity of SSB in transcription, the authors suggested that SSB may be acting as a subunit of the N4 RNA polymerase, although, again, there is no direct evidence for a physical association.

**Modulation of Enzymatic Activities by SSB**

The activities of many enzymes are either stimulated or inhibited by the presence of SSB. Proteins that must bind ssDNA to carry out their function are often inhibited by SSB, since such proteins have a lower affinity for ssDNA and cannot displace bound SSB. Many DNA-dependent ATPases that require a single-strand effector show inhibition of the ATPase activity by SSB and include Rep, n′ (except on φX174 DNA), ATPase IV, RecA, helicase II, DnaB, and a variety of mammalian enzymes (8, 13, 90, 95, 186, 245, 247, 256, 258, 262, 352, 382, 383). Transcription is inhibited by SSB (288), as are most single-strand exo- and endonucleases (131, 234, 258, 270–272, 327, 387). Indeed, protection of ssDNA may serve as one of the major functions of SSB in vivo. In these cases, the effect is most probably mediated through interactions of SSB with ssDNA rather than any direct effect on the nucleic acid. For exonuclease I (272) and the 3′→5′ exonuclease activity of polymerase II, which are stimulated by SSB (2, 122, 271, 272). Supercoiled DNA contains single-stranded regions that are susceptible to S1 nuclease. Binding of SSB facilitates this digestion (137). These regions may be important for initiation of transcription or recombination. SSB prevents binding of the carcinogen N-acetoxy-2-acetylaminofluorene, an inhibitor of DNA synthesis (37). The F sex factor of E. coli carries a gene, psiB, which inhibits SOS induction, and the inhibition is enhanced by the F SSB protein (140). Several other enzymes are stimulated by SSB, including the helicase activity of DNA helicase II (245), topoisomerase I (371), gene A protein of φX174 (390), the RecA ATPase at high Mg2+ concentrations (54, 56), E. coli DNA polymerase II (45, 270, 356), and the replication activity of polymerase III holoenzyme (249). DNA polymerase I and the DNA polymerase III core enzyme are inhibited by the DNA-binding protein (112, 113, 249, 270, 271, 356), and the 3′→5′ exonuclease activity of DNA polymerase III holoenzyme is inhibited under replication conditions but is not affected in the absence of replication (355). The T7 DNA polymerase is stimulated by SSB (126, 272, 283, 284, 314, 337, 376).

**INTERACTIONS OF SSB WITH NUCLEIC ACIDS**

Since the discovery of the phage T4 gene 32 protein and E. coli SSB by the nature of their strong affinity for ssDNA, these proteins have been extensively studied from the point of view of protein-nucleic acid interactions. As it is the intention of this review to provide an overall view of the varied aspects of E. coli SSB, these interactions will be considered here. However, more detailed physiochemical analyses of DNA binding, which is beyond the scope of the present review, have appeared in recent years, and the reader is directed to these reviews for more in-depth discussions (76, 91, 133, 188, 220, 221, 419).

**Helix Destabilization**

Although SSB belongs to the group of proteins known as helix-stabilizing proteins (5), the term is misleading (76). It
should be reiterated that SSB cannot completely denature native dsDNA at physiological ionic strength or temperature. At low ionic strength (2 mM Tris hydrochloride [pH 8.1], 1 mM trisodium EDTA), slow denaturation of T4 dsDNA was observed by Sigal et al. (356); however, others, using T7 dsDNA, were unable to reproduce these findings (423). Even at 150 mM NaCl, no denaturation of T7 DNA was observed (423). Supercoiled DNA generally contains some localized regions of destabilized helix to which SSB can bind and promote the loss of supercoils (137, 203). Helix-destabilizing ability can be demonstrated as a decrease in the helix-coil transition temperature of poly(dA-dT). Normally poly(dA-dT) melts at 66°C in 150 mM NaCl. In the presence of SSB, the Tm is reduced to 57.5°C. For comparison, the proteolytic fragments of SSB reduce the Tm even further to 51°C (SSBF) and 46.5°C (SSBC) (423). The binding proteins isolated from ssb mutants (discussed below) show a decrease in helix-destabilizing ability in one case (SSB-1 [422]) and an increase in the other case (SSB-113 [72]). Once hydrogen bonds of a double helix are destabilized spontaneously, by heat, or by a DNA helicase, the single strands will be stabilized by SSB. (The interaction of SSB with DNA helicases is discussed in the sections on DNA replication and DNA repair.)

There is one report that SSB can promote the renaturation of denatured DNA (81). The rate of renaturation of λ DNA was enhanced ca. 5,000-fold by SSB, but only in the presence of spermidine or spermine and saturating amounts of SSB. The significance of this reaction remains unknown.

Cooperative Binding

E. coli SSB can bind cooperatively to single-stranded polynucleotides. Cooperativity is the phenomenon whereby an SSB tetramer has a higher affinity for a polynucleotide or a segment of a polynucleotide to which an SSB is already bound than to one lacking SSB. Cooperativity of SSB was first demonstrated by electron microscopy (356) which showed that at limiting protein concentrations, SSB molecules were unevenly distributed, with some portions of the DNA containing clusters of SSB and others lacking SSB (83, 149, 151, 326, 356). Cooperativity has also been demonstrated by a variety of other techniques including gel filtration (404), nuclease digestion (340), density gradient centrifugation (83, 356), fluorescence quenching (224, 273), filter binding (340, 423), agarose gel electrophoresis (224), and nuclear magnetic resonance spectroscopy (149).

Cooperativity can be expressed by the cooperativity parameter, ω, a dimensionless constant characterized by the equilibrium between an SSB bound singly on a polynucleotide lattice and one bound adjacent to another protein. Estimates of ω have ranged from 50 (149) to 10 (326). However, there are a number of inherent difficulties with these measurements, such as artifacts of the technique itself, limits of sensitivity, potential errors associated with the experimental conditions, and theoretical models used to analyze the data (220). Although it is clear that SSB does bind cooperatively to ssDNA, there is no general agreement as to the magnitude of ω.

More recently, Lohman and co-workers (61–63, 219, 224) have distinguished two types of cooperativity: unlimited nearest-neighbor cooperativity, reflecting interactions on either side of bound SSB tetramers, and limited cooperativity, which is limited to the formation of nucleosome-like octamers. The limited binding cooperativity appears to form in the (SSB)4 tetrameric binding mode (224) (see below).

Since an SSB molecule has four DNA-binding sites, it is appropriate to ask whether all four contact the DNA. Experiments addressing the question are normally carried out with synthetic homopolymers to avoid any secondary structure that could affect the measurements. Using equilibrium dialysis, Ruyechan and Wetmur (327) concluded that only two binding sites per tetramer were used. Using ultracentrifugation with various oligonucleotides to assess binding, Krauss et al. (191) found that SSB could bind four d(pT)8, two d(pT)16, and one d(pT)30. Since all four binding sites could potentially be bound to polynucleotide, the authors proposed that the DNA wraps around the SSB tetramer (see the section on nucleosome-like structures below). In subsequent experiments with fluorescence quenching, stoichiometries of 4, 2, 2, 1, and 1 for d(pT)16, d(pT)28, d(pT)35, d(pT)46, and d(pT)50, respectively, were reported (61, 62). The differences in values have been attributed to differences in the sensitivities of the techniques (220). Bujalowski and Lohman (61) have recently described a phenomenon of negative cooperativity among the multiple binding sites, in which the binding of an oligonucleotide at one site negatively influences the binding at a second site on the tetramer. Although all four binding sites appear to be equivalent, a nonequivalence between dimers within the tetramers occurs upon binding of the initial sites to ssDNA. This negative cooperativity is strongly affected by salt, suggesting that it involves electrostatic repulsion (62). It is postulated that such negative cooperativity contributes to the different binding modes that SSB can assume with ssDNA (220).

Kinetics of DNA Binding and Transfer

The SSB-ssDNA complex is very stable. Kinetic analyses of the dissociation of SSB from oligo d(pT)30–40 in a noncooperative binding mode indicates a koff of <1 molecule/s (191, 322). Since DNA replication proceeds at a rate of 500 to 1,000 nucleotides/s, dissociation and/or transfer of SSB tetramers must be facilitated in some way. Römer et al. (322), using 1H nuclear magnetic resonance spectroscopy, have observed a high rate of translocation of SSB which cannot be accounted for by a simple association-dissociation phenomenon. They propose that SSB translocates by a walking or sliding mechanism. Schneider and Wetmur (340), using different methods, also concluded that some sort of direct transfer of intact tetramers between DNA strands was occurring. Although experiments of this sort provide some useful information about the behavior of the isolated protein and nucleic acid in vitro, the situation at the replication fork is far more complex. If free SSB is in excess, it is more likely that newly denatured single-stranded regions will be bound by SSB from the free SSB pool (340). Estimates of the amount of SSB in the E. coli cell and the amount of ssDNA at replication forks (43) would argue that a significant pool of free SSB does exist. Moreover, models of association-dissociation kinetics fail to consider possible protein-protein interactions such as that of DNA polymerase III holoenzyme with SSB, which may promote dissociation of SSB in advance of the replicating polymerase. Therefore, the walking of SSB tetramers down the single-stranded ladders or the transfer of intact SSB tetramers between strands may not have any biological relevance.

Nucleosome-like Structures

The fact that SSB appears to bind DNA in a tetrameric form has been addressed earlier in this review (section on
The general properties. The protein domains involved in DNA binding have also been discussed. Sigal et al. (356) first observed that upon binding of SSB, the contour length of phage fd DNA decreased by ca. 35%, corresponding to a spacing of 0.18 nm per nucleotide. Subsequent microscopy studies by Griffith and co-workers (83, 150, 151) indicated that the DNA may take on a beaded appearance that was dependent upon the protein/DNA ratio. At low ratios the complexes appeared as partially opened clusters; at intermediate ratios they appeared as open chains of beads and protein-free linkers; at high ratios closely juxtaposed distinct beads were observed; and at very high ratios (8:6:1) the contour length increased abruptly and the protein appeared as a smooth-contour form. In the high-density beaded mode, the M13 DNA had a contour length only 20 to 24% that of the naked DNA, whereas in the very high density, smooth-contour mode, the DNA length was 38 to 42% that of native ssDNA (151). Greipel et al. (149) also observed a beaded appearance of SSB bound to poly(dT) with a contour length ca. 25% that of unbound poly(dT).

The interpretation of these data is that the DNA is wound around SSB, as first suggested by Krauss et al. (191). The difference in appearance has been attributed whether the DNA is wound around an octamer (beaded) or a tetramer (smooth contour). Nuclear magnetic resonance spectroscopy (89) and nuclease digestion (83) have provided further support for a histonelike nucleosome binding of SSB. Limited digestion of SSB-complexed phage fd ssDNA with micrococcal nuclease resulted in a regular banding pattern of 160 ± 25 nucleotides, and extensive digestion resulted in a single band at 145 nucleotides. In the electron microscope, the DNA, after extensive digestion, had the appearance of single beads. Data from equilibrium density banding of formaldehyde-glutaraldehyde-fixed, nuclease-digested DNA suggested the association of an octamer of SSB subunits per 145-nucleotide segment, with approximately 30 nucleotides of protein-free linker DNA between octamers. Further studies with DNase I digestion revealed a repeating banding pattern starting at 25 bases and then spaced at 15-base intervals. This pattern is consistent with the notion that DNA is wrapped around a protein core with the edge of each monomer providing a site for DNase I attack (83). Boidot-Forget et al. (44), using nuclease P1 digestion of SSB-bound poly(dT), observed a banding pattern of ca. 80 nucleotide repeats. The difference between these findings and those of Chrysogelos and Griffith (83) may have resulted from differences in the nuclease used, the DNA substrate [fd DNA versus poly(dT)], the NaCl concentration, the divalent cations present, and the temperature of incubation (220).

Whether SSB binds to ssDNA as an octameric structure has been examined by Lohman and co-workers (59, 61, 62, 294). By measuring binding to homopolymers in solution, the cooperativity between adjacent tetramers to form octamers was found to be only moderate (294). Octamer formation is also dependent upon protein-binding density (59, 83, 151). Therefore, when SSB is bound to nucleic acid, not all tetramers will necessarily be associated into octamers.

Variations in DNA-Binding Modes

Each SSB monomer has a potential DNA-binding site. Early estimates of the binding site size, n, covered by a single SSB tetramer when bound to a nucleic acid lattice were in the range of 30 to 36 nucleotides (191, 273, 326, 356, 404), although values as high as 70 were reported (9, 404). The first evidence that there may be variability in binding modes came from electron spin resonance spectroscopy studies of spin-labeled polynucleotides (43). Shortly thereafter, salt-dependent binding modes of 33 ± 3 and 65 ± 5 were observed by fluorescence quenching (223). Later, the same group reported four distinct modes of 35 ± 2, 40 ± 2, 56 ± 3, and 65 ± 3, observed over a range of MgCl₂ and NaCl concentrations (58), and, more recently, binding modes of 35, 56, and 65 have been discussed (63). These binding modes are strongly dependent upon monovalent and divalent salt concentrations, temperature, pH, and protein-binding density (58, 63, 223). Moreover, they are observed when a polynucleotide lattice is added to a solution of SSB (reverse-titration conditions). In contrast, Bobst et al. (submitted) observed by using electron spin resonance spectroscopy and a regular-titration protocol (i.e., addition of SSB to a lattice in solution) that the binding stoichiometry was 60 to 70 and was independent of the NaCl concentration. A regular-titration approach provides a more straightforward analysis than does a reverse-titration approach (60). When reverse titrations were carried out at low salt concentrations (5 mM NaCl), a metastable, low-nucleic-acid-density binding stoichiometry of 25 to 30 was found. This could easily and quickly be converted to a 60 to 70 stoichiometry by addition of more NaCl (Bobst et al., submitted).

There is always a danger in attempting to extrapolate in vitro data on the binding of SSB and synthetic homopolymers to what actually occurs in vivo on a stretch of ssDNA. At present there seems to be a general agreement that SSB, under presumably physiological NaCl and SSB concentrations, will bind a DNA lattice with n = 65 ± 5 for a tetrameric stoichiometry. At high SSB and low ssDNA concentrations, which may exist in the cell, an (SSB)₅₅ mode could form. Whether or not the other binding modes, (SSB)₉₀ and (SSB)₁₆₀, would actually occur in vivo is questionable. Furthermore, suggestions that the different binding modes may be used for different functions, such as replication versus recombination (220), seem speculative in the absence of direct experimental data.

Binding Affinities for Various Polynucleotides

There is no general agreement on the absolute values of affinity constants for SSB binding to polynucleotides. Such differences can be attributed to the different methods used to collect the data, theoretical modeling, and variations in the assay conditions used. Such factors as salt concentrations, temperature, and lattice size can influence these parameters (see reference 220 for a detailed discussion). By using oligonucleotides, the affinity for d(T)₃₀₋₄₀, for example, was estimated at 5 × 10⁸ M⁻¹ (191) and the affinity for poly(dT) was 10⁶ to 10⁷ M⁻¹ (223, 273). E. coli SSB shows great variability in its affinity for different homopolymers and natural DNAs. Although absolute binding constants are difficult to obtain, there have been some determinations of relative affinities, Kₚₛₛₛ. By using an electron spin resonance competition method developed to determine relative binding affinities for gene 32 protein (42), the following relative affinities were obtained for SSB: Kₚₛₛₛ(dA₃₀₋₄₀) ≈ 4Kₚₛₛₛ(dT₃₀₋₄₀) = 40 Kₚₛₛₛ(dA₃₀₋₄₀) = 200 Kₚₛₛₛ(dT₃₀₋₄₀) (Bobst et al., submitted). Overman et al. (294), using fluorescence quenching (0.20 M NaCl at 25°C and pH 8.1, i.e., the (SSB)₅₅ mode) obtained the following hierarchical values for the intrinsic association equilibrium constants (Kₒₛₛ): Kₒₛₛ(dT) > Kₒₛₛ(dC) > Kₒₛₛ(dG) > Kₒₛₛ(ssM13 DNA) > Kₒₛₛ(I) > Kₒₛₛ(U) = 8Kₒₛₛ(dA) = 8Kₒₛₛ(dA) (A). Considering the different methods and strategies used, the qualitative ranking between the two
approaches is fairly good. Both studies show that SSB has greatest affinity for poly(dT) compared with other polynucleotides, with natural DNA binding with less affinity.

**ssb gene**

**Genetic Studies**

Identification of _ssb_ gene. The _ssb_ locus was first identified in 1979 during a screening of _dna_ mutations which had not as yet been identified with a specific gene product (136, 257). Severstopoulos et al. (345) had isolated a number of _dna_ mutants and identified two new loci, designated _dnaI_ and _dnaM_. Strain SG1635 was reported to carry the temperature-sensitive mutation _dnaM710_. Extracts of this strain failed to support G4 DNA replication in vitro even at 30°C unless supplemented with SSB, although radioimmunoassays indicated the presence of cross-reacting material to anti-SSB antibody. In assays with purified replication proteins and SSB from these cells, replication occurred at 30°C but not at 42°C, thus identifying the defect as a temperature-sensitive SS B (257). The original _dnaM710_ mutation had been mapped to 74 to 79 min on the old _E. coli_ map (345); however, the SG1635 strain actually contained two temperature-sensitive mutations. One mapped at ca. 75 min and was temperature sensitive at 41°C, but not defective in DNA replication. The second mutation mapped at 90.8 min and was temperature sensitive at 42.5°C and defective in DNA replication (136, 257). This mutation was designated as _ssb-l_ (257) and allowed the mapping of the _ssb_ gene (136). Since 1979, the _E. coli_ map has been expanded to 100 min and map positions have been revised accordingly. In Fig. 5 a current map in the region of the _ssb_ gene at ca. 92.1 min is shown.

**ssb mutations.** The first mutation in _ssb_ was actually discovered by Greenberg and colleagues in an _E. coli_ B strain in 1974 and was designated _exxB_, as it was extremely sensitive to γ radiation and to alkylating agents such as 1-methyl-3-nitro-1-nitrosoguanidine, methyl methanesulfonate (MMS), and ethyl methanesulfonate (EMS) (145, 148). The mutation was mapped to the _malB_ region and was shown to be distinct from another radiation-sensitive mutation in this region, called _exrA_ (lexA). The _exxB_ mutation differed from _exrA_ in several aspects. One included filamentation in a _lon_ background (145, 162). The second was that _exxB_ cells were temperature sensitive for DNA synthesis, whereas _exrA_ cells were not (147). This mutation was subsequently renamed _lex-l13_ (106). Johnson (162) made a detailed map of this area of the _E. coli_ chromosome by using the _lex-l13_ mutation. He then renamed it _lexC113_, after demonstrating that it was not a _lexA_ mutation. On the basis of the similarities in defects described for the _lexC113_ and _ssb-l_ mutations (136, 145, 147, 257), we considered that _lexC113_ may be a second _ssb_ mutation (258). This was confirmed by demonstrating that SSB isolated from _lexC113_ cells had a temperature-sensitive SS B (259). This mutation was subsequently renamed, and it is now known as _ssb-l13_ (259).

All of the known _ssb_ mutations are listed in Table 3. Of these, _ssb-l_ and _ssb-l13_ have been the best characterized, and their physiological effects will be discussed in detail below. No information is available on _ssb-2_. The _ssb-3_ mutation was discovered by Schmellick-Sandage and Tessman (339a; I. Tessman, personal communication). This mutation renders the cell extremely sensitive to UV irradiation, with a survival curve similar to that for a _recA_ mutation. However, _ssb-l3_ has only a slight effect on DNA replication (Meyer and Laine, unpublished). This mutation will undoubtedly prove very useful in uncoupling the effects of SSB on DNA replication from those on DNA repair and in identifying the protein domains involved in DNA repair. Johnson (164) described a new mutation designated _ssb-l14_, obtained by nitrosoguanidine mutagenesis. Cells carrying the _ssb-l14_ mutation were reported to be more sensitive to UV irradiation and to the alkylating agent MMS and showed other differences when compared with _ssb-l13_ cells (164). However, Chase and Williams (76) noted that the _ssb-l14_ mutation carries the identical amino acid change to the _ssb-l13_ mutation, i.e., Pro-176→Ser. Since there are marked physiological differences between the two mutations in an apparent isogenic background (164), the most likely explanation is that _ssb-l14_ carries a second mutation at another site within the _ssb_ gene, particularly since the parent strain had been highly mutagenized. However, it is also possible that there is a mutation in another gene closely linked to _ssb_ that is involved in DNA repair (such as _uvrA_) and that the results reported by Johnson (164) are due to this double mutation. In vitro studies of _SSB-l14_ or complete sequencing of _ssb-l14_ should resolve this question.

Chase and co-workers (65, 172) have used site-directed mutagenesis to produce several mutant proteins with specific amino acids changes as listed in Table 3. These were used to probe the functional domains of SSB as discussed earlier in this review (section on functional domains). Recently Porter et al. (299) have described the construction of an _E. coli_ strain with the _ssb_ gene deleted. This strain survives only by carrying an _ssb_ plasmid.

**Physiological effects of _ssb_ mutations.** The _ssb_ mutations exhibit a variety of phenotypic effects which will be briefly described here. Most _ssb_ mutants are temperature sensitive for DNA replication (136, 257, 258). They are defective in a variety of DNA repair processes, being UV sensitive (136, 164, 211, 339a, 411), defective in _RecA_ induction (29, 164, 263), Weigle reactivation (411), and mutagenesis (164, 211, 411), and showing excessive postirradiation DNA degradation (29, 211). There is an increase in transposon excision in an _ssb_ background (232). Recombination deficiencies of _ssb_ mutants and mutant proteins have been noted in vivo (110, 136, 141) and in vitro (247). Therefore, all aspects of DNA metabolism, i.e., replication, repair, and recombination, are affected. In later sections these effects will be discussed in further detail.

**Physiological suppression of _ssb_ mutations.** The temperature-sensitive phenotype due to the _ssb-l_ and _ssb-l13_ mutations can be physiologically suppressed in several ways. Lieberman and Witkin (210) were the first to observe that expression of the defect in DNA replication of _ssb-l_ could be corrected simply by transducing the mutation into an _E. coli_ B background. However, the defect in DNA repair was not affected. Tessman and Peterson (380) made a careful study of suppression of both the _ssb-l_ and _ssb-l13_ alleles under a variety of conditions. In a strain _Cl_a background, both temperature sensitivity and UV sensitivity of the _ssb-l_ mutation were partially suppressed. This suppression was shown to be due the wild-type _rep_ gene (_rep_c1a) carried by this strain, since transduction of _rep_c1a_ into an _E. coli_ K-12 background also suppressed _ssb-l_. It should be noted that _rep_c1a_ is not defective in Rep helicase activity; i.e., it does not produce a Rep phenotype, yet clearly it differs from other wild-type Rep proteins. The concentration of NaCl in the culture medium also greatly influences the suppressibility of _ssb-l_ as determined by the efficiency of plating at high temperature compared with 30°C. In a detailed analysis of...
Transposon insertions also by high salt concentrations. The suppression, sensitivity of strains occurs only in rich medium, whereas Wang and Smith (401) found that both ssb-1 and ssb-113 cells exhibited greater UV sensitivity when plated on a rich medium than on a minimal medium. Salt suppressibility of temperature-sensitive mutations in E. coli has been observed with many alleles and is thought to be due to an interaction of electrolys with mutant proteins (176). In the presence of high-ionic-strength buffers, hydrophobic interactions would be favored. For ssb-1, this may stabilize the tetramers, whereas for ssb-113, this may favor protein-protein interactions that are normally disrupted at 42°C.

Genetic suppression of ssb mutations. Suppresser analysis is a powerful genetic tool, as it can reveal new genes affecting a given metabolic process and it can identify hitherto unsuspected protein-protein interactions. The theory of suppressor analysis has been discussed in detail previously (46, 146, 154). Greenberg et al. (146) examined the reversion frequency of exrB (ssb-113) mutants compared with wild-type cells either on MMS or at 42°C. They reported a spontaneous reversion frequency of $10^{-4}$ to $10^{-5}$ in an E. coli B background. This is considerably lower than a rate of $10^{-4}$ to $10^{-5}$ that we have observed in a K-12 background (S. E. VanDenBrink-Webb, D. C. Rein, and R. R. Meyer, unpublished data). However, these values are strongly influenced by the genetic background of the strains, culture medium used, and amount of salt on the plates (P. S. Laine and R. R. Meyer, unpublished data). As these mutations may be physiologically suppressed, it is necessary to distinguish between true genetic suppression and physiological suppression. All revertants of ssb-113 showed a simultaneous resistance to UV irradiation, MMS, and a growth temperature of 42°C (146). The authors also reported two classes of revertants: those that could be cotransduced with malB and those that could not. Cotransduction with malB indicates that these could be true revertants, i.e., lying within the ssb gene, or that they could map near the ssb gene. No further characterization of these revertants has been reported.

Donch and Greenberg (106) reported that the lexA102 mutation could suppress filamentation of lex-113 (ssb-113) seen in lon strains. It also had a small effect on suppressing the UV sensitivity of lex-113 cells.

Johnson (163) made the interesting observation that a mutation of phage P1 called lsc can suppress ssb-1, ssb-113, and ssb-114 mutations for both temperature and UV sensitivity. Phage P1 may lysogenize in E. coli cells at a low frequency. It does not become integrated into the chromosome but, rather, is maintained as an episome. These results strongly suggest that phage P1 encodes its own single-stranded DNA-binding protein, which may be used for E. coli chromosomal replication. This interpretation is supported by the observation that the F factor ssb gene (177) was discovered by the ability of strains carrying F to suppress temperature-sensitive growth of ssb-1 cells. Other plasmid ssb genes were subsequently found by using this complementation test (142, 143). The plasmid ssb genes were also shown to suppress UV sensitivity. These data would argue that the plasmid-encoded SSB is fully functional and can substitute for chromosome SSB in E. coli DNA replication and repair. Therefore, one mechanism of suppression is (It should be recalled that overproduction of SSB-1 completely suppresses all polyphenotypic effects of ssb-1 [74].)

An effect of the growth medium on the expression of ssb defects has also been reported. Lieberman and Witkin (210) observed that salt suppression of ssb-1 strains occurs only in rich medium, whereas Wang and Smith (401) found that both ssb-1 and ssb-113 cells exhibited greater UV sensitivity when plated on a rich medium than on a minimal medium. Salt suppressibility of temperature-sensitive mutations in E. coli has been observed with many alleles and is thought to be due to an interaction of electrolites with mutant proteins (176). In the presence of high-ionic-strength buffers, hydrophobic interactions would be favored. For ssb-1, this may stabilize the tetramers, whereas for ssb-113, this may favor protein-protein interactions that are normally disrupted at 42°C.

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to provide a protein than can substitute functionally for the *E. coli* SSB.

We have isolated 51 independent, spontaneously arising suppressors of ssb-1 and 8 of ssb-l13 (260, 325; VanDen-Brink-Webb et al., unpublished). Genetic analysis indicated that none of these are true revertants or intragenic suppressors. The first suppressor examined in detail was shown to be a mutation of groEL (groEL11), the major heat shock protein of *E. coli* (286, 287), and a protein essential for growth of λ and other phages (124). An interesting pattern of DNA synthesis was observed upon temperature shift in ssb-1 groEL11 cells (Fig. 6) (260, 325). Immediately upon shift to 42.5°C, the rate of DNA synthesis fell by 70%, but within 4.5 min the cells recovered and continued synthesis at a rate parallel to that of control cells (Fig. 6). It has been suggested that groEL11 protein may promote functional SSb-1 tetramer formation at the nonpermissive temperature, either by a direct interaction with groEL11 protein and SSB-1 or by an increase in the local concentration of SSB-1 monomers to shift the equilibrium to tetramers (260). Radioimmunossays indicate that cells carrying the groEL11 allele have higher levels of SSB-1 protein (ca. 30% higher at 32°C and ca. threefold higher at 42.5°C) (260). However, the kinetics of recovery after temperature shift would argue against a rapid synthesis of SSB-1 as the mechanism of suppression.

**Cloning of the ssb Gene**

Shortly after the discovery of the ssb gene, Sancar and Rupp, who were attempting to clone the uvrA gene, reported construction of a plasmid (pDR2000) carrying both the uvrA and ssb genes (330). Although these two genes lie very close to one another (see the section on ssb regulation below), the cloning of the intact ssb gene was fortuitous, as EcoRI was used to obtain the insert and the ssb gene contains an internal EcoRI site that should have been cleaved if the reaction had gone to completion. Although cells carrying multicopy plasmids with the ssb gene overproduce SSB 5- to 300-fold (72, 74, 75, 222, 263), such overproduction surprisingly does not have much effect on the physiology of the cell, except that such cells are partially altered in their DNA repair capacity (52, 209, 263, 275, 277, 328, 385). The ssb gene has been cloned in several laboratories, mostly as an aid in obtaining large quantities of the protein (3, 24, 38, 50–53, 72, 74, 75, 222, 330, 333, 341, 422, 427). Cloning of the ssb gene and placing it under control of the λ p1 promoter lead to yields as high as 3 mg of SSB per g of cells (222).

Scholteske and Grosse (341) have recently used a novel system to clone the ssb gene fused to collagen, which could then be released by treatment with collagenase. The procedure is not needed for overproduction of SSB, but was used to demonstrate the general applicability of the vector system.

**Sequence of the ssb Gene**

The sequence of the ssb gene was first reported by Sancar et al. (333), with minor corrections noted by Chase et al. (72). The entire sequence is shown in Fig. 1; the regulatory sequences are discussed in detail below (see Fig. 7). The coding sequence is 534 nucleotides in length, although the initiation methionine does not appear in the final SSB protein (333). A termination codon follows the Phe-177 residue. The ssb gene does not have a conventional termination structure, and there is no sequence homology to ρ-dependent termination sequences (333). The ssb gene is transcribed clockwise on the standard *E. coli* map (Fig. 4). One unusual feature is that the ssb gene lies very close to the uvrA gene.

**Regulation of the ssb Gene**

**Quantitation of SSB levels in the *E. coli* cell.** Estimates of the amount of SSB in *E. coli* have relied upon quantitation of specific activity of SSB on SDS-polyacrylamide gel electrophoresis (23, 102, 296) or competitive radioimmunoassay to detect levels of SSB in soluble extracts (72, 74, 260, 329, 392, 404). Values ranging from ca. 300 to 2,000 tetromers per cell have been reported (43, 72, 74, 260, 296, 329, 392, 404). There are two explanations for these discrepancies. When SSB is bound to DNA, the efficiency of detection is reduced by a factor of 2 (43). Unless precautions are taken to eliminate DNA or to dissociate SSB from the DNA, for example, by running the assays in 2 M NaCl (43), underestimates of SSB levels may occur (72, 74, 404, 422). Second, the growth conditions of the culture also have an effect, as cells grown in rich media have higher levels of SSB than the same cells grown in minimal media (43). This may simply reflect the fact that faster-growing cells have more replica-
tion forks. The presence of other mutations also can affect SSB levels. Arnold et al. (23), using two-dimensional gel electrophoresis to quantitate protein levels, observed a 30% decrease in SSB in strains carrying the rho-15 mutation. Different strains were also found to vary in the amount of SSB (43), and basal levels of SSB fall as the culture goes from log to stationary phase (275). On the basis of these observations, the wild-type E. coli cell in mid-log phase is estimated to contain 1,000 to 2,000 SSB tetramers, depending on the strain and growth conditions (43).

Levels of SSB in mutant cells. Several estimates of the level of SSB-1 and SSB-133 determined by radiolabelling have been published, but with inconsistent results (72, 74, 260, 392, 422). Chase et al. (74) found levels of SSB-1 to be the same as or slightly higher than those of SSB, whereas Villani et al. (392) found no significant differences. Meyer et al. (260), on the other hand, reported levels of SSB-1 to be reduced. The physiological significance of this is difficult to assess, given the uncertainties of the radiolabelling assay and the variability seen as a function of genetic background, culture conditions, and growth rate (43). Since sbb-1 cells grow more slowly than sbb+ cells, this may itself account for some of the decrease.

With respect to sbb-113 strains, Chase et al. (72) found radiolabelling values of SSB-113 to be ca. 30% lower than those of SSB and attributed this to a reduced affinity of SSB-113 protein to the polyclonal antibody prepared against SSB+ . However, Villani et al. (392) found no differences in values for SSB and SSB-113.

Translational control. It has long been known that the gene 32 protein of bacteriophage T4 binds its own mRNA and regulates translation of this protein (208). Thus far, there is only a single report to suggest that SSB may interact with its own mRNA (350). Such binding led to an inhibition of translation in a cell-free system at SSB concentrations above 0.5 μM (350), the estimated physiological concentration of this protein (422). The idea of autoregulation of SSB is intriguing. An increase in ssDNA levels would occur by initiation of new replication forks in response to transfer to richer media or by inoculation of stationary-phase cells into fresh media. Since the binding of SSB to ssDNA would be greatly favored over the binding to mRNA, SSB would be titrated off the mRNA, resulting in an increased translation of SSB-specific mRNA. However, establishing whether SSB does autoregulate its own level in a manner analogous to gene 32 protein (as was elegantly demonstrated by Lemaire et al. for the T4 DNA-binding protein [see reference 208 and references therein]) will require much more rigorous analysis than that provided (350).

Figure 7. Regulatory region of the sbb and uvrA genes of E. coli. The figure has been drawn from published sequences (26, 50, 51, 332, 333). The two structural genes and direction of transcription are shown in brackets at the top left and bottom right of the figure. Since transcription occurs in opposite directions (open arrows), only the sbb strand is shown. The −35 and −10 sequences of uvrA and the three sbb promoters are shown in boxes, connected by a solid line above or below the sequence. The transcriptional starting points are indicated by the solid arrows. The LexA-binding sequence (SOS box) is indicated by a double line and brackets.
sequence of this inducible ssb promoter lies only seven nucleotides from the −35 sequence of uvrA and only two nucleotides from the upstream limit of the LexA repressor recognition sequence. Therefore, it is not surprising that the proximity of the PI promoter to the SOS box would lead to suppression of transcription from this ssb promoter under noninduced conditions. Upon DNA damage and LexA repressor cleavage, transcription from PI occurs. However, a careful examination of the in vivo transcripts before and after SOS induction (see Fig. 3 in reference 51), indicates that efficient transcription from the PI promoter is accompanied by a dramatic decrease in transcription from promoters P\text{N1} and P\text{N2}.

Whether ssb is inducible under SOS repair conditions has also been examined in several other laboratories (3, 275, 296, 329, 392, 411). By radioimmunoassay, no increase in SSB accumulation was detected (296, 329, 392, 411). Alazard (3), using a different approach, introduced cloned ssb and uvrA genes into lexA3 (Ind−) cells which are noninducible for SOS. Although these studies were not quantitative, they showed that synthesis of UvrA was suppressed while SSB was not. These results are in apparent contradiction to those of Brandma et al. (50, 51), who used gene fusion. It should be noted that the latter authors did not measure the levels of SSB directly but, rather, measured the levels of galactokinase. To resolve this discrepancy, Perrino et al. (296) measured both the relative rate of synthesis and accumulation of SSB. Although the level of SSB in the extracts did not rise over a 1-h period after SOS induction, there was a slow increase in the relative rate of synthesis of SSB, indicating that ssb is inducible but leads to no significant rise in total SSB levels. Moreau (275) has reported about a twofold increase in SSB levels, but only after a 3-h exposure to very high doses of mitomycin C. These results do not contradict those of Perrino et al. (296), as Moreau did not begin to see any accumulation of SSB until after 1 h posttreatment, whereas Perrino et al. terminated their experiment at 1 h.

Although it appears that ssb is, indeed, inducible, this induction is very slow relative to that of other genes in the recA-lexA SOS regulon (396, 397). Therefore, it is questionable whether it has any functional significance for SOS repair.

**ROLE OF SSB IN DNA REPLICATION**

In this section we shall address specifically the role of SSB in various in vitro replication systems. It is not our intent to provide a comprehensive review of DNA replication, as this has been discussed in detail in numerous other reviews to which the reader is directed (10, 115, 179, 181-185, 242, 248-251, 291, 292, 384, 394, 416). Moreover, as SSB has been used almost universally in in vitro replication assays for many years, we have not attempted to cite all such references, but only those of historical relevance or those providing insight into the mechanism(s) of action of SSB.

**SSB Is Required for DNA Replication In Vivo and In Vitro**

In vitro DNA replication systems were established in the early 1970s by using ssDNA phage templates (ΔX174, M13, fd, Φλ, G4, φ3, ST-1, and φK) for SS→RF synthesis (242). From the beginning, SSB was always a standard component of the assays (34, 134, 335, 404, 415, 417, 429), yet there was no direct evidence that SSB was essential in vivo. Indeed, under certain conditions, SSB could be eliminated from the in vitro assays and replication would still proceed (11). Moreover, some DNA polymerases such as polymerase I and polymerase III (core enzyme) were strongly inhibited by SSB (112, 113, 249, 270, 356). The essential role of SSB in DNA replication could not be unequivocally established until the discovery of an ssb mutation (257). Strains carrying ssb− were temperature sensitive for DNA synthesis in vivo, with replication terminating within 1 to 2 min at 42.5°C (Fig. 6) (136, 260, 325). This indicated that ssb− was a quick-stop mutation and was involved in chain elongation. At the nonpermissive temperature, in vivo replication of phage was also impaired (136). In vitro assays with SSB prepared from mutant cells demonstrated this temperature sensitivity (257-259) and, indeed, showed that the temperature sensitivity of SSB−1 protein was completely reversible (Fig. 4) (258).

**SSB Organizes and Stabilizes DNA Replication Origins**

The first evidence that SSB promotes the formation of structures that permit initiation at replication origins in vitro came from studies of phage G4 replication (48, 415). The phage G4 system is the simplest, requiring only SSB, primase, and DNA polymerase III holoenzyme for SS→RF synthesis. Primase cannot bind specifically and stoichiometrically to the G4 origin unless the template is saturated with SSB (373). Sims and Benz (357), using the related phage φK, found that primase did not bind at all to φK ssDNA in the absence of SSB, confirming similar observations reported earlier by Wickner (415) for G4. Stayton and Kornberg (373) found ca. 15 primase molecules bound per G4 single-stranded circle in the absence of SSB, but such binding was abolished by addition of MgCl₂. Only in the presence of SSB were stoichiometric amounts of two primase molecules per circle observed. Both the sequence in the region of the replication origin (114, 358) and the ca. 28-nucleotide primer synthesized at this origin in vitro in the absence of DNA polymerase III holoenzyme (47) predict a secondary hairpin structure. Additional secondary structure occurs within the first 106 nucleotides of the origin (373). Sims and Benz (357) showed by nuclease protection studies with φK DNA that primase protects two hairpin sequences, one at the origin and one further downstream. They suggested that both secondary and tertiary structures are involved in the formation of an origin signal and that such nucleic protection is absolutely dependent on the presence of SSB. It is likely that primase interacts with SSB. The transcript begins six nucleotides upstream from the base of the hairpin, and as the primase proceeds into the secondary structure, SSB promotes destabilization of the helix so that primase may synthesize a cRNA primer.

Initiation of φX174 DNA replication requires the formation of a primosome at the replication origin (12, 15, 353). Other primosome assembly sites have also been found in pBR322 (431), CoElI (289, 290, 431), and mini-F (161). As with the G4 origin, the primosome assembly sites involve secondary and tertiary structures, as evidenced by the fact that such sequences have little homology with one another (243) and that deletions (368) or mutations (1, 144, 366) in these sequences inactivate primosome-dependent DNA replication. Apparently, SSB can melt out such mutated sites while stabilizing those with a native form (144). Primosome assembly is discussed in detail in the section below.

Initiation of complementary-strand replication for filamentous phages such as M13 and fd involves the use of RNA polymerase instead of primase to synthesize a primer (55, 132, 134, 167, 242, 391, 414). Specificity of initiation for these phage templates is likewise provided by SSB. The RNA is
similarly transcribed from a hairpin loop, and the sequence is protected by RNA polymerase from nucleosome digestion, but only in the presence of SSB (132, 334). The σ subunit is required for specific recognition of the initiation structure (167).

The origin of *E. coli* chromosomal replication (oriC) has been cloned on plasmids and used as a substrate to determine the events involved in initiation (128–130). Initiation first requires the binding of DnaA (127). DnaC complexes with DnaB and delivers it to the initiation complex (130), with the subsequent release of DnaC (395). The initiation complex is stabilized by SSB. The template is unwound by the helicase action of DnaB and DNA gyrase in the presence of SSB (27, 28) in preparation for the priming event (389).

Phage λ requires SSB for replication. Initiation of replication from a double-stranded supercoiled plasmid containing the λ origin requires X0 and λP proteins in addition to seven *E. coli* host proteins (253). The initiation event is started by the binding of X0 protein to the λ origin to which λP and *E. coli* DnaB proteins then associate in a nucleoprotein complex (105). Subsequently, DnaJ, DnaK, and SSB bind in the presence of ATP; this leads to an unwinding of the helix at the origin (104, 251). There is an absolute requirement for SSB which serves both to stimulate DnaB helicase activity and to stabilize the origin for the subsequent priming event (251).

Phage T7 induces its own DNA-binding protein, which has been purified and characterized (313, 314, 337). However, unlike the other T phages, T7 may be able to use the host SSB during replication in vivo. In fact, mutations in the T7 binding protein (gene 2.5 protein) were identified by the inability of such phages to grow on *E. coli ssb* mutants (21). The ability of SSB to substitute for T7 binding protein in DNA replication assays in vitro has been known for some time (126, 264, 283, 284, 314, 315, 320, 321, 336, 337). It functions in initiating bidirectional synthesis at the T7 replication origin (126). In addition, SSB promotes strand displacement, although it is not as effective as the T7 binding protein in promoting the multiple initiations along the lagging strand (284).

**Formation of the Primosome and Its Interaction with SSB**

The primosome is a complex of several proteins which act as a mobile priming apparatus in the SS→RF replication of ϕX174 (10, 12, 15, 182, 252, 261). Figure 8 shows the steps involved in complementary-strand synthesis. Protein n' (also known as factor Y [417]) is the key component, as it recognizes the hairpin loop formed at the origin of replication (351). Binding of a single n' molecule permits an ordered assembly, with binding of proteins n and n' (15, 228). This is followed by binding of DnaB. However, DnaC must first form a complex with DnaB in the presence of ATP and Mg²⁺ (175, 395, 418). It serves to escort DnaB to the assembling primosome, where, in the presence of DnaT (formerly called protein i), DnaB binds, with the release of DnaC (17, 395). This complex is called a prepriming complex and can be isolated as a stable unit. Addition of primase completes the formation of the primosome (15). The primosome translocates in a 5'→3' direction, with primers synthesized at several sites (12, 16). DNA polymerase III holoenzyme elongates the primers; DNA polymerase I excises the RNA and fills the gaps, which are subsequently sealed by DNA ligase to complete the SS→RF phase (Fig. 8) (353). The primosome remains attached to the RF I form and can be used for subsequent priming in a reaction coupling SS→RF and RF→SS synthesis (20).

The *E. coli* SSB plays an important role in SS→RF replication by directing the assembly of the primosome at the ϕX174 replication origin. Upon binding, protein n' displaces some SSB molecules, and additional SSBs are displaced upon primosome assembly (Fig. 8) (13, 352). Binding of protein n in the assembly process requires n' to be bound first and can occur only in the presence of SSB. As indicated above, in the section on protein-protein interactions, protein n is one of the few proteins for which a direct physical interaction with SSB has been demonstrated (228). As the primosome translocates 5'→3' along the DNA, more SSB is dissociated so that the template may be read by primase. As the DNA polymerase III holoenzyme extends these primers, the remaining SSBs are dissociated in advance of the polymerase (Fig. 8).

Recent models of the replication fork have proposed that DNA polymerase III holoenzyme functions as a dimer that couples leading-strand and lagging-strand replication by looping the lagging strand through the polymerase (184, 249). It has been suggested that the two DNA helicases, DnaB with 5'→3' polarity and n' with 3'→5' polarity, which are stimulated by SSB, could provide a coordinated action to feed this DNA loop, with the n' helicase displacing SSB and relieving secondary structures of the lagging-strand template (204).

**SSB Is Required for Priming Specificity**

*E. coli* DnaB is essential for DNA replication; it is a part of the primosome, and it is a helicase (205). On any naked ssDNA and in the absence of other prepriming proteins, DnaB can function as a mobile promoter signal by translocating down the DNA, and, by itself, organizing secondary structures that can be recognized by primase to synthesize a short RNA primer (11, 14). This type of initiation has been termed the general priming reaction (11). Such reactions do not require SSB and are characterized by the synthesis of numerous short primers distributed all around the circular template. The addition of SSB to such replication systems suppresses the general priming reaction in favor of synthesis of a unique primer at or near the in vivo origin of replication for G4, ϕX174, and M13 (12, 15). Thus, another essential role for SSB in replication is to ensure specific initiation at the proper origin. It should also be recalled that SSB similarly provided transcriptional specificity for phage N4 RNA polymerase (324).

**Helix Unwinding Requires Coordinated Action of SSB and DNA Helicases**

Helix-destabilizing proteins are characterized by their ability to decrease the helix–random-coil transition temperature of duplex DNA (5). Although SSB can destabilize a DNA helix (356), it cannot do so at physiological temperatures and under ionic conditions (251). DNA helicases are capable of translocating along a helix and disrupting hydrogen bonds, driven by the hydrolysis of ATP. However, in the absence of SSB, renaturation rapidly occurs. The unwinding of DNA at replication origins and the subsequent translocation of DnaB helicase are greatly stimulated by both DNA gyrase (27) and SSB. It is presumed that binding of the latter protein to ssDNA, to prevent reannealing, drives the reaction (205). Similarly, Rep, whose helicase action is required during RF→SS replication of ϕX174 (18, 19, 109, 311, 344) and possibly *E. coli* chromosome replication, is also stimulated by SSB. The primosomal protein n' is also a helicase.
SSB Enhances Fidelity of DNA Synthesis

The fidelity of DNA replication has been a topic of intense study for the past 20 years (125, 192, 218). There are many agents which will decrease replication fidelity, but only two activities have been shown to enhance fidelity. The 3'→5' exonuclease activity associated with certain DNA polymerases has a pronounced effect on reducing misincorporation by virtue of a proofreading function. It was surprising to find that SSB also enhances the fidelity of a variety of procaryotic and eucaryotic DNA polymerases by as much as an order of magnitude when measured on synthetic polynucleotides, natural DNA substrates, or depurinated DNA (193, 194). Enhanced fidelity was observed even with polymerases that were inhibited by SSB and that contained no 3'→5' proofreading exonuclease activity (193). Therefore, the mechanism of enhancing fidelity cannot be simply explained through stimulation of a 3'→5' exonuclease. It has been suggested that SSB enhances base selection through increased rigidity of the template, which, in turn, allows the polymerase to better determine whether there is a correct fit with the incoming nucleotide before a phosphodiester bond is formed with the 3'-OH of the primer. Depurination of DNA increases mutagenesis. Addition of SSB to fidelity assays with depurinated φX174 DNA resulted in a two-
fivefold decrease in mutagenesis, but did not completely eliminate it (194). Further support for a role of SSB in replication fidelity comes from the observation that ssb mutations promote an increase in spontaneous mutagenesis, as discussed in more detail in the section on DNA repair.

Michaels et al. (264) have shown that DNA polymerase I can synthesize past mutagenic acetylaminofluorene adducts. However, in the presence of SSB, such lesion bypass is suppressed. These observations add further support to those of Kunkel et al. (193, 194) that SSB enhances the fidelity of DNA synthesis.

SSB Enhances Processivity of DNA Polymerases

The *E. coli* DNA polymerase III holoenzyme consists of at least 7, and probably as many as 13, different subunits (238–240, 249). Four distinct subassemblies of the holoenzyme may be isolated or reconstituted that are capable of DNA synthesis, but only the holoenzyme form can replicate ssDNA phage and *E. coli* plasmids containing the origin of replication (oriC) in vitro (249). Bambara and co-workers (112, 113, 197, 199, 200) have made extensive studies of the processivity of the holoenzyme and its subassemblies. Processivity is a measure of the average number of nucleotides inserted by a polymerase per template-binding event. The DNA polymerase III core enzyme has a limited processivity of 10, whereas the holoenzyme can completely replicate a 5,577-nucleotide G4 ssDNA template in a single binding event (112, 197, 200) or more than 100,000 nucleotides in a rolling-circle, strand displacement mode of synthesis by using a model template (269). The other subassemblies, DNA polymerase III' and DNA polymerase III*, have intermediate processivity values of 60 and 200, respectively (113). Interestingly, SSB enhances the processivity of the more complex forms (polymerase III* and the holoenzyme), but has no effect on polymerase III' and inhibits polymerase III core enzyme (112, 113) or core enzyme plus β subunit (199). Therefore, the biologically relevant form (i.e., the holoenzyme) requires SSB for its high processivity and may, in fact, be capable of replicating the entire *E. coli* leading strand with a single binding event if SSB is present (249).

Using DNA sequencing gels, LaDuca et al. (200) identified pause sites for DNA polymerase III holoenzyme and its subassemblies. These pause sites, which terminated synthesis, were correlated with positions of potential secondary structure. In the presence of SSB, the high-molecular-weight subassemblies and the holoenzyme could progress through these sites, indicating that removal of secondary structure by SSB is the probable mechanism of enhancement of processivity. Similar effects of SSB on DNA polymerase II had been noted earlier by Sherman and Gefter (347) and have also been reported for phage T7 polymerase (376). That melting of secondary structure is one of the mechanisms of T7 polymerase stimulation has been confirmed by Myers and Romano (283).

SSB Promotes Polymerase Binding

*E. coli* SSB has a second effect on the T7 DNA polymerase in addition to melting hairpin loops on the template. By using substrates with and without potential secondary structure, Myers and Romano (283) observed that SSB still stimulated the polymerase, with the greatest stimulation at low polymerase concentrations. The binding protein was shown to promote productive binding of the polymerase to the polynucleotide substrate when the polymerase was limiting. A similar enhancement of polymerase II binding to DNA by SSB had been demonstrated earlier (271, 272).

Summary of Roles of SSB in Replication

In summary, SSB plays a central and varied role in DNA replication: (i) it enhances helix destabilization by helicases; (ii) it prevents reannealing of the single strands and protects against single-strand nuclease digestion; (iii) it helps to organize and stabilize replication origins; (iv) it is required for primosome assembly; (v) it ensures the specificity of priming; (vi) it enhances the fidelity of the DNA polymerase; (vii) it enhances the processivity of the polymerase by destabilizing secondary structure that could cause polymerase pausing and dissociation; and (viii) it may promote binding of the polymerase to the template.

ROLE OF SSB IN DNA REPAIR

*E. coli* has several pathways for the repair of DNA damage. It is beyond the scope of this review to summarize these pathways except for those in which there is evidence for participation of SSB. DNA repair has been reviewed numerous times in the past 10 years (36, 88, 123, 153, 195, 213, 214, 267, 268, 276, 307, 331, 396–399, 407). As indicated earlier in this review, the first evidence that SSB is involved in DNA repair came from the observation that ssb mutants are UV sensitive (136, 145, 164, 211, 339a, 411). Subsequent studies have provided evidence that SSB plays a role in methyl-directed mismatch repair, the SOS response, and recombinational repair, but seems to have little, if any, role in other DNA repair processes.

SSB Is Required for Methyl-Directed Mismatch Repair

Replication errors which are not corrected by the 3'→5' exonuclease proofreading activity of DNA polymerase III holoenzyme ε subunit (338, 339) may result in a base-pair mismatch. The methyl-directed DNA mismatch correction system normally corrects such errors before they are fixed as mutations in the DNA. This repair process exploits the fact that DNA methylation at d(GATC) sites is a postreplication event and that the strand containing the replication error will be undermethylated. Genetic studies have shown that there are several mutator genes, identified by the fact that mutations in these genes result in an elevated spontaneous rate of mutagenesis (268). These genes include the following: dam, the DNA methylase responsible for methylating d(GATC) sequences; mutD (identical to dnaQ), which encodes the ε subunit of DNA polymerase III holoenzyme; mutH; mutL; mutS; mutU (identical to uvrD), which encodes DNA helicase II; and mutY. In recent years considerable progress has been made in establishing in vitro systems for mismatch repair (201, 229, 230; for reviews, see references 88, 267, 268, and 307). The first reconstitution of a complete mismatch repair system with purified enzymes in vitro was reported within the past year (201). This system is dependent upon eight proteins: MutH, MutL, MutS, DNA helicase II, SSB, DNA polymerase III holoenzyme, exonuclease I, and DNA ligase. By using phage fl heteroduplex molecules, a single-base-pair mismatch located within overlapping recognition sites for two restriction enzymes was constructed. Such a substrate is refractory to cleavage by either enzyme, whereas correction of one strand will generate a restriction site for one endonuclease but correction of the opposite strand provides a recognition sequence for the
other. The substrate molecule also contains a single d(GATC) methylation site. Mechanistically, first, MutS is thought to bind to a mismatch site (375). Subsequently, MutM, which has endonuclease activity, cleaves 5′ to the dG base of an unmethylated d(GATC) site, in the presence of Mg2+, to produce 3′-OH and 5′-PO4 termini. MutM may also sequester the break to prevent religation. MutL appears to activate MutH nuclease in the presence of MutS and ATP (152), which suggests that binding of MutS facilitates the binding of MutL and then MutH (268). Since the incision site may be 1,000 nucleotides or more from the damage site, it is not clear how the signal is transduced from MutS to MutH.

SSB is thought not to play any role in this initial phase, but rather to function in the excision and resynthesis step (268). Two different models have been proposed, although neither has been confirmed experimentally. The first model, presented by Modrich and co-workers (230, 268, 374, 375), proposes that repair synthesis begins at the break produced by MutH and suggests the possibility that this protein facilitates the entrance of the excision-repair proteins. It is presumed that resynthesis proceeds 5′→3′. The helix is unwound by helicase II translocating 3′→5′ on the template strand (244), driven by SSB binding to the single strands (245), followed by resynthesis by DNA polymerase III holoenzyme. Whether this involves a strand displacement mechanism has not been determined, although in the absence of DNA synthesis large gaps are formed (374). Exonuclease I has recently been identified as the 55-kDa stimulatory protein required for in vitro mismatch repair (201). Since exonuclease I is a 3′→5′ single-strand-specific exonuclease and is stimulated by SSB, it would suggest that exonuclease I hydrolyzes the strand in which the misincorporated base is located. How a 3′ terminus is provided for this enzyme, however, is not known. In contrast, Langle-Rouault et al. (202) proposed that the event is initiated at the mismatch where helix distortion, perhaps aided by MutS and/or MutL, could provide an entry point for helicase II, with helix destabilization proceeding in both directions if a helicase is bound to each strand.

What is the role of SSB in this process? In vitro methyl-directed mismatch repair has an absolute requirement for SSB (201, 230). Three key enzymes in mismatch repair interact functionally with SSB: DNA helicase II unwinding is driven by SSB; exonucleolytic excision of the displaced, error-containing strand by exonuclease I is stimulated by SSB; and repair resynthesis of the strand over a distance of as many as several thousand nucleotides is effected by DNA polymerase III holoenzyme, requiring SSB bound to the template strand, as discussed above in the section on DNA replication.

**SOS Response**

**ssb mutants are defective in RecA induction.** The SOS response to DNA damage in E. coli is mediated through the recA-lexA regulon (reviewed in references 215 and 396 to 399). The lexA gene encodes a repressor which binds the operators of at least 17 different genes called damage-inducible (din) genes (398). Interruption of replication or damage to the DNA generates an induction signal, which activates the constitutive level of RecA, which in turn promotes cleavage of LexA. Depending upon the level of LexA and the relative affinity of LexA for the various operator sequences, increased expression of these genes occurs. Several SOS-induced genes, including uvrA, uvrB, uvrC, uvrD, recA, and recN, are involved in error-free DNA repair. In addition, umuC, involved in error-prone (mutagenic) repair is induced. The net result is an increase in overall repair capacity. The key step in this process is the induction of RecA protein. The UV sensitivity of ssb mutants has been attributed in part to the failure to induce normal levels of RecA (29, 164, 211, 263). There are, however, some discrepances in the literature with regard to the levels of RecA induction. Baluch et al. (29) reported that RecA induction by UV in ssb-1 cells was normal at 30°C but absent at 42°C and that ssb-113 cells failed to induce RecA protein at any temperature. Bleomycin, however, was an effective inducer in ssb-1 and ssb-113 strains at both 30 and 42°C. Meyer et al. (263) found reduced levels of RecA synthesis in both strains and at both temperatures whether using UV or nalidixic acid as inducer, and Lieberman and Witkin (211) observed normal levels in both strains at 30°C and "substantial levels" at 42°C when using nalidixic acid. Johnson (164) carried out his experiments only at 37°C and reported reduced levels in ssb-1 cells and a lack of induction in ssb-113 strains after UV treatment. Both strains, in contrast, induced RecA with nalidixic acid. Although quantitative differences may be attributed at least in part to differing genetic backgrounds and the induction conditions used, it is clear that the induction process is impaired in ssb mutants. SSB is undoubtedly involved in the activation of RecA. This is supported by the observations of Meyer et al. (263) that thermal induction of RecA in a recA441 (rif-1) background is unaffected by ssb-1. This was confirmed by Whittier and Chase (412) and extended to include ssb-113 strains. Overproduction of SSB-1 by a multicycop plasmid can restore to normal levels the induction of RecA in ssb-1 cells with a concomitant decrease in UV sensitivity (74).

**SSB promotes RecA-dependent cleavage of LexA and λ repressors.** Since cleavage of LexA is the critical step in RecA induction, this raises the question of the role of SSB in the process. Induction of the SOS regulon leads to cleavage of a variety of lysogenic phage repressors including λ, P22, 434, and φ80 (212, 317, 398). The cleavage site for both LexA and λ repressors is at an Ala-Gly bond, located in a protease-sensitive hinge region (360). Cleavage of these repressors is autocatalytic (212, 360). It is now thought that RecA acts indirectly to stimulate this reaction as an allosteric effector when bound to LexA or λ repressors (407). For RecA to promote this cleavage, it must be in an activated form which requires two cofactors: a nucleoside triphosphate and either an ssDNA or a UV-irradiated DNA (100, 101, 231, 297). What role does SSB play in this activation process? In vitro SSB stimulates RecA'-mediated (312, 406) or RecA441-mediated (278) cleavage of λ repressor. By using SSB-113, cleavage of λ repressor at low SSB-113 concentrations was stimulated, but it became inhibitory at higher levels (312). Cohen et al. (70) observed that SSB-113 competed more effectively than SSB with RecA for binding ssDNA. This is supported by the observations of Chase et al. (72) that SSB-113 has a higher affinity for ssDNA than SSB does. This may subsequently prevent the activation of RecA protein and thereby reduce the level of LexA or λ repressor cleavage.

**Lambda phage induction in ssb mutants.** Lambda phage requires SSB for DNA replication in vitro (see the section on DNA replication), and cleavage of the λ repressor is promoted by RecA activation, a function defective in ssb cells. Using UV as an inducing agent, Vales et al. (388) reported that ssb-1 strains had a 5-fold reduction in λ yield at 30°C, but a 10-fold reduction at 42°C. The ssb-113 (lexC-113) strain showed a 250-fold reduction at 30°C and a 100-fold reduction...
at 42°C. Qualitatively similar results were obtained with mitomycin C treatment (388). Bleomycin treatment, which can induce RecA in ssb strains, also led to a corresponding increase in yield of λ* (291). Johnson (164) also examined λ induction in ssb strains at 37°C and found reduced levels of λ* in ssb-1 cells, but the effect was only about 5-fold for UV and 3-fold for nalidixic acid induction, compared with the 103-fold effect reported by Vales et al. (388) at 37°C. Strains carrying the ssb-113 or ssb-114 mutation showed no UV induction of λ* phage (164).

That the defect lies in the induction process was confirmed by using λ cI857 prophage and heat inactivation of the repressor. Under these conditions phage yields in the mutants approached that of wild-type cells (388). Moreover, in a recA0281I background (constitutive for high levels of RecA) both the spontaneous and induced levels of phage were higher in ssb-1 and ssb-113 strains. Overproduction of SSb-1, which restores the ability to induce RecA and suppresses UV sensitivity, also abolishes the defect in λ prophage induction in ssb-1 cells (74).

It is interesting that when λ cI857 was induced in ssb-1 cells at 42°C and incubation was continued at this temperature, no reduction of λ titers was observed. Furthermore, incorporation of labeled thymidine into λ DNA continued at 42°C, while E. coli chromosomal DNA replication was impaired (388). These results appear to be in contradiction to the in vitro studies on λ DNA replication (discussed in the section on DNA replication), for which SSb is required. It suggests that either (i) λ DNA replication in vivo can bypass the requirement for SSb; (ii) the λ phage can sequester enough SSb-1 protein, perhaps by raising the local concentration or by interaction with a phage protein and thereby providing functional SSb-1 tetramers; or (iii) SSb-1 monomers may be used. This phenomenon warrants further investigation.

**Effects of ssb mutations on rates of spontaneous and induced mutagenesis.** Greenberg et al. (146) were the first to examine mutagenesis in eexB (also known as ssb-13) cells and found no enhanced mutagenesis after treatment with MMS. Whittier and Chase (411) examined the frequency of λ clear-plaque mutants with and without UV irradiation of the host cell. At 30°C they observed no difference in the spontaneous mutagenic rate of ssb-1 or ssb-113 (lexC113) cells. Upon UV irradiation of the host, there was an increase in the number of clear plaques in ssb-1 cells, but this was only half that seen in ssb+ controls. In contrast, ssb-113 cells showed no change. Interestingly, at 42°C the spontaneous rate for ssb-1 cells was nearly fourfold higher than that of ssb+ cells, whereas the ssb-113 rate was less than half that of controls. Both ssb-1 and ssb-113 strains showed an increase in induced λ phage mutagenesis after host irradiation, but the levels were only 42 and 8.6%, respectively, of that of ssb+ controls.

Lieberman and Witkin (210, 211) have made a careful study of mutagenesis in ssb strains. When transduced into an E. coli B background, ssb-1 mutants are no longer temperature sensitive, and such strains could be used to examine UV-induced mutagenesis at both 30° and 42°C. At 30°C the yield of streptomycin-resistant mutants was normal, but it was reduced fivefold at 42°C in ssb-1 strains (210). By pretreatment of these cells with nalidixic acid at 30°C to induce RecA prior to temperature shift, UV-induced mutagenesis could be restored to control levels (211). In examining ssb-113 strains, these authors (211) reported a 10-fold increase in spontaneous mutagenesis of trp to trp*, although there was no effect on UV-induced mutagenesis. Using other markers (6-azauracil resistance and mutagenesis of argE to argE*) Quiñones and Piechocki (302) reported elevated levels of spontaneous mutagenesis (2-fold for ssb-1 and 10-fold for ssb-113). These values correlate well with those of Johnson (164), who found 3-fold (ssb-1), 11-fold (ssb-113), and 13-fold (ssb-114) increases. In contrast to the findings of Lieberman and Witkin (211), Johnson observed UV-induced mutagenesis with all three mutations, although the relative increase was far smaller than in wild-type strains. In SOS constitutive cells (recA730), both the spontaneous and induced mutagenic rates were comparable to those in controls (211).

These studies indicate that ssb-1, ssb-113, and ssb-114 strains have a 2- to 13-fold higher rate of spontaneous mutagenesis. In view of the fact that SSb enhances replication fidelity, as discussed above (section on replication fidelity), decreased fidelity in ssb mutants could easily explain these findings. The lack of or reduced level of induced mutagenesis, however, can be attributed solely to the failure to induce the SOS response (211).

**Decreased Weigle reactivation in ssb mutants.** Walker has compiled a list of 27 different physiological responses to SOS induction in E. coli (398). Apart from RecA induction, phage λ induction, mutagenesis, and induction of SSb itself (discussed in the section on the regulation of the ssb gene), few other SOS responses have been examined in ssb mutants. One of these is Weigle reactivation.

Weigle reactivation (403) is the enhanced survival of UV-irradiated phage that occurs if the host cell is first irradiated at low doses to induce the SOS response. Whittier and Chase (411) found that at 30°C, a threefold activation in ssb-1 strains occurred immediately after irradiation, but it did not increase further after 30 min; little or no reactivation occurred in ssb-113 cells. In control cells under the same conditions, 9- and 25-fold activation was observed at these times. At 42°C ssb-1 cells showed no UV reactivation. Although these data clearly demonstrate that Weigle reactivation is impaired, it is surprising that the authors did not find higher levels of reactivation in the ssb-1 strain at 30°C, since they had previously reported normal RecA induction in ssb-1 cells at this temperature (29). In recA441 (iss-l) strains in which RecA can be thermally induced in an ssb-1 or ssb-113 background (263, 412), Weigle reactivation was normal (412).

**Enhanced DNA degradation in ssb mutants.** SSb mutants have a high rate of DNA degradation at both 30 and 42°C after UV irradiation (2, 29, 400, 411-413). Such DNA degradation with ssb-1 cells was observed even when the mutation was transduced into a genetic background in which DNA replication and growth can occur at 42°C (211). Hyperdegradation was also found in a recA0281I background, in which there is a high constitutive level of RecA protein (211, 412). It was also observed in ssb-1 recC22 cells in which the RecBCD exonuclease (exonuclease V) is inactivated (211). The RecBCD nuclease is known to cause DNA degradation in recA mutants (233). These data imply that SSb-1 may be less efficient in protecting ssDNA from such degradation (211), and, indeed, such decreased protection from nuclease attack in vitro with SSb-1 protein has been demonstrated (258). These data also indicate that another nuclease(s) may participate in this DNA degradation, such as exonuclease I or the 3’→5’ exonuclease activity of polymerase II which are both stimulated by SSb (2, 122, 271, 272). Double-strand breaks also accumulate in ssb mutants (400).

**SSB and recombinational repair.** When the presence of a DNA lesion, such as a pyrimidine dimer or bulky adduct,
prevents reading of the template strand by DNA polymerase III holoenzyme, the polymerase may dissociate and reinitiate replication at the next Okazaki fragment, leaving a single-stranded gap (318, 354). This may be repaired through a recombinational event, with subsequent repair of the lesion by an excision repair mechanism. Recombinational repair is error free. *E. coli* SSB is required for recombination, and this will be discussed in detail below (section on SSB in recombination). However, it should be recalled that the *ssb* mutants are defective in recombination (110, 136, 141). This may simply reflect a decreased efficiency of mutant SSB interacting with RecA (247). Therefore it is likely that the UV sensitivity of *ssb* mutants can be attributed to three factors involved in recombinational repair: (i) the inability of the mutants to induce sufficient levels of RecA protein for enhanced recombination; (ii) a decreased efficiency of RecA-SSB interaction during the recombination process itself, leading to an accumulation of ssDNA; and (iii) the inability of mutant SSB to adequately protect against DNA degradation. It is the last result that ultimately leads to cell death.

**Effects of SSB Overproduction on DNA Repair Processes**

Surprisingly, the *E. coli* cell can tolerate a large overproduction of SSB provided by multicopy plasmids carrying the *ssb* gene, although there are some physiological effects associated with this. Such cells grow at a slightly reduced rate (S. M. Ruben and R. R. Meyer, unpublished observations). We first reported that cells overproducing SSB were moderately UV sensitive (263), an observation confirmed by Bransdina et al. (52). Such strains were able to induce only about half the normal level of RecA after UV irradiation or treatment with nalidixic acid. It was speculated that overproduction of SSB may interfere with activation of RecA (52, 263). This has been examined in more detail by Moreau (275), who reported that excess SSB leads to a decrease in LexA repressor concentration with a simultaneous increase in basal levels of two lexA-controlled genes: recA by 5-fold and sfi by 1.5-fold. The sfi gene could be fully induced after DNA damage, but required a higher level of inducing agent. Although there was evidence of increased efficiency of excision repair, recombinational repair was reduced (275, 277), and this probably accounted for the UV sensitivity reported by us and others (52, 263, 275, 277). Moreau (276) has proposed that overproduction of SSB may not interfere with activation of RecA, but, rather, may delay the formation of long nucleoprotein filaments of RecA, which in turn reduces the rate of recombinational repair. As the physiological effects of overproduction of SSB mimic those seen in recF cells, Moreau (277) has further suggested the possibility that RecF protein helps RecA protein displace SSB from ssDNA during recombination.

Recently Lerš et al. (209) reported that overproduction of SSB enhances photorepair, but only in *recA* cells. These authors suggested that SSB binds the ssDNA regions formed by distortion of the double helix about the pyrimidine dimers, which in turn stimulates photolyase. They have also shown that overproduction of SSB allows continued DNA synthesis in *recA* cells after UV irradiation, whereas in cells containing only a chromosomal copy of *ssb*, DNA synthesis is blocked (385). This was interpreted that SSB may aid in transducer DNA synthesis.

**ROLE OF SSB IN RECOMBINATION**

**Overview**

*E. coli* SSB provides an important adjunctive function in recombination. The literature contains divergent opinions about the exact roles and interactions of SSB in the molecular mechanisms of recombination, and we have attempted to present a balanced view in this review article. In recent years, numerous reviews on this subject have included discussions of the roles of SSB. The reader is directed to these reviews for a more detailed analysis of recombination (96, 150, 187, 304, 306, 361–363, 381, 405, 407).

Considerable progress has been made in the past decade toward an understanding of the molecular mechanisms of recombination. Clark (85) originally proposed that recombinational events may be viewed as part of one or more pathways, similar to the intermediary metabolism pathways of the cell. Since several of the intermediates and enzymes of recombination remain unknown, these pathways have been defined largely on the basis of genetic analysis (363). The first recombination-deficient mutants were isolated by Clark and Margulies in 1965 (86) (see the review by Smith [361] for details).

The principal pathway of homologous recombination in *E. coli* is the RecBCD pathway (361). Two other pathways have been identified, the RecE and RecF pathways, although they are not as well understood. All three share the requirement of RecA. This 38,742-Da single polypeptide is a DNA-dependent ATPase that promotes, in vitro, a set of DNA strand interactions which mimic its in vivo functions in a homologous recombinational event (93, 95, 99). Mutational studies indicate that RecA is essential for recombination, except when the RecE pathway operates on plasmid-plasmid and lambda-lambda DNA heteroduplexes (346) and when the lambda Red pathway is used (306).

The major focus of this first section is to examine how SSB functions in relationship to the recombinase activities of RecA. Later the role of SSB in each of the three pathways of recombination, RecBCD, RecE, and RecF, will be examined.

**SSB-RecA Interaction**

In vitro recombination. It is worthy at the outset to mention two pertinent facts: (i) at present there is no direct evidence for a physical interaction between SSB and RecA (187, 221); and (ii) a general problem in the study of recombination is the lack of good cell-free systems as are available for replication (407). Thus far in vitro recombination systems are difficult to reproduce reliably, or they appear to promote recombination by artificial mechanisms (362). Many data have been reported to suggest that these two proteins do, indeed, affect each other's activities in recombination. A schematic diagram of the recombination process is shown in Fig. 9.

Most investigators divide the molecular events of in vitro recombination into three phases: presynapsis, synapsis, and strand exchange (96, 306, 362). Following the formation of a right-handed helical nucleoprotein filament, a search for homology begins. This results first in a nucleoprotein complex or network (aggregation) and then in a pairing of the ssDNA to a complementary area of the duplex DNA, called homologous alignment or D-loop formation. The synapsis event initiates a unidirectional reciprocal strand exchange reaction (strand invasion, strand assimilation) coupled with
ATP hydrolysis (301). This is distinct from branch migration, which occurs after the classic DNA intermediate, the Holliday structure (306). One end product of this strand exchange process is a heteroduplex DNA molecule.

The in vitro role of SSB has been described as both auxiliary (305) and critical (187). Most evidence indicates that SSB functions nonspecifically to stimulate RecA interactions with ssDNA in the formation of a nucleoprotein filament (92, 190, 280), i.e., presynapsis. However, some recent evidence suggests that the presence of SSB may also be needed to permit RecA to function effectively during the entire DNA strand exchange process (Fig. 9) (190, 280, 424).

Presynaptic events. (i) Formation of the nucleoprotein filament. Data from in vitro assays of RecA activities have provided most of our understanding of homologous recombination. The pairing of circular ssDNA (M13, φX174, G4) with nicked linear duplex DNA has provided an ideal system to evaluate the phases of the strand exchange process (303).

The requirements for presynapsis are RecA, ssDNA, and ATP. Evidence for a requirement of SSB is inconclusive. Although SSB seems to participate (280, 369, 370), others have reported that it was not essential under a variety of conditions tested (39, 69, 107, 372) but that it accelerated the reaction (57, 67, 99, 190, 247, 386). Inhibition occurs in the presence of low concentrations of ADP (168, 281). Other factors affecting this reaction are the concentration of Mg$^{2+}$ ions, temperature, type of DNA substrate used (190), and the order of addition (57, 67, 120).

The formation of nucleoprotein filaments is almost completely inhibited at 30°C unless SSB is added, and at 37°C SSB prevents their decay (57, 168). SSB will not only counteract the inhibitory effects of low temperature, but also permit filament formation in the presence of ADP (168). If SSB and RecA are added simultaneously to ssDNA at 37°C, a competition is noted with RecA having a higher affinity for ssDNA than SSB has if ATP is present, but a lower affinity...
if no ATP is added (119). RecA displaces SSB from ssDNA (319). Fluorescence and kinetic experiments revealed a replacement of 40% of RecA from the ssDNA substrate after the addition of SSB and ATP (92). These data suggest that SSB may be part of the nucleoprotein filament; however, no stable associations could be demonstrated (369). Flory and Radding (118) reported differences in the appearance of RecA-ssDNA complexes formed in the absence and presence of SSB, although there was no direct evidence that SSB was in the filament. Recently, however, Heuser and Griffith (156), have shown by electron microscopy that RecA and SSB bind simultaneously to M13 ssDNA (Fig. 9).

Tsang et al. (386) have reported the isolation of SSB-containing DNA complexes by gel filtration after the simultaneous addition of RecA and SSB. The filaments contained 1 molecule of RecA per 3.6 nucleotide residues and 1 molecule of SSB per 15 nucleotide residues. It has been proposed that SSB might influence the binding of RecA protein to ssDNA by ordering the DNA in some manner (92). From subsequent electron-microscopic studies (151), which allowed visual examination of the ternary complex, it was suggested that SSB may be an assembly factor for RecA in its binding to ssDNA and that structural alterations in the ATP-primed RecA protein occur after association with ssDNA and SSB. This causes a nucleation event, resulting in a very ordered filament (151). Using a negative staining procedure, Williams and Spengler (424) have noted distinctly separate regions on the filament where the two proteins are bound. Fluorescence quenching has also shown an ATP-dependent interaction between SSB and RecA-ssDNA complexes (280).

When SSB and ATP are both available, the binding of RecA seems to be unidirectional with a 5'→3' polarity (310). Circular dichroism studies indicated that RecA can be bound in more than one conformation to its DNA substrate (432). These experiments again suggest a true physical interaction between SSB and RecA, which may occur only when both are bound to ssDNA.

Binding stoichiometries (287) indicated that the amount of RecA complexed to ssDNA was linearly dependent on the concentration of SSB. One SSB monomer was required for the binding of five RecA monomers, until a concentration was reached corresponding to one SSB monomer per 20 nucleotides of ssDNA. At this concentration, the complex appeared stable and could be analyzed after gel filtration (287). This stability seems to be maintained by SSB, which prevented the dissociation of RecA from the ssDNA (99), although this has been disputed by others (189). If SSB was omitted, self-aggregation and renaturation of the single strands occurred (306). The presence of SSB made these polymeric complexes of RecA and ssDNA kinetically competent as intermediates in the next phase of the strand exchange process (95, 168, 279). There are two views which could explain how a binding protein might help in stabilization of the filament. SSB could help RecA to patch up sites where the filament has decayed, or SSB could play a more structural role by maintaining a precise conformation for the nucleoprotein filament (306).

Specificity of the reactants. The question still remains whether SSB or other DNA-binding proteins are absolutely required for the presynaptic stage. It has been argued that SSB simply melts secondary structure from the single strands of DNA, which impedes the binding of RecA during the presynaptic phase (190, 281, 409). Thus, it is a facilitator for the association of RecA (120) as well as a stabilizer (97, 280). These facilitation processes are not specific to SSB, but can be accomplished by a variety of other DNA-binding proteins including the T4 gene 32 protein, the λ B protein, SSBs encoded by F' and conjugative plasmids, and the variant SSBc (107, 280, 281). In vitro conditions which favor the removal of hairpins in the ssDNA also expedite the binding of RecA to its ssDNA substrate (159, 168, 281).

Egner et al. (107) claim that specific interactions of SSB with RecA play no important role in presynaptic polymerization. In contrast, when West et al. (410) purified the RecA protein of Proteus mirabilis (RecApm) and examined how it interacted in an in vitro assay with SSB of E. coli, recombination was markedly altered. RecApm was unable to produce a productive nucleoprotein filament to serve as a stable intermediate for phase 2. Therefore, SSB may be interacting physically and species specifically with RecA when both are bound to ssDNA (90).

Recently a RecA mutant protein which is recombinase deficient but retains an unchanged ssDNA-dependent ATPase function has been purified (56). In the presence of SSB, the ATPase activity was strongly suppressed (56). By using normal RecA under appropriate Mg2+ conditions, this activity, as noted above (i) was stimulated. These results also suggest that physical contact by SSB may be needed to induce a conformational change in RecA. It would be interesting to see whether suppressors of the recA mutation could be found which map in ssb, or vice versa.

(ii) Stability of the nucleoprotein filament. The degree to which SSB remains associated with the active RecA nucleoprotein filament has been the subject of intense debate. If this association is indeed real, SSB may directly enhance the cooperative interactions in the filament complex (287). Hydrolysis of ATP occurs throughout the nucleoprotein filament and is stimulated by SSB (54). Data from a DNase I nuclease protection experiment showed 100% protection with both proteins present in the assay, whereas only 80% protection was observed for each protein separately (301).

There is considerable evidence that SSB is required for subsequent strand exchange events (54, 168, 180, 280, 287, 301, 310, 369, 408). The exchange process was at least twofold more efficient in the presence of SSB (98, 168), and in the presence of an acetate buffer a three- to fourfold increase was reported (319). SSB is stable for up to 1 h (280) and probably continues to function throughout strand exchange (369). Complexes isolated on sucrose density gradients contained RecA bound to the heteroduplex DNA product and SSB bound to the displaced ssDNA (369); they could be observed in the electron microscope (118, 309, 372).

Synaptic events. (i) Homologous pairing. If SSB is required in subsequent molecular activities which culminate in recombination, what functions might it contribute? The homologous pairing event in the majority of in vitro studies involves the formation of a junction between the circular ssDNA and linear duplex DNA. These junctions were visualized by electron microscopy as loop formations (246, 348). The pairing seems to occur in a biphasic reaction with paracentric joints preceding plectonemic joints (120, 316). The paracentric joint does not require a free homologous strand of DNA and shows no net cross-strand interwinding. On the other hand, plectonemic joints are resistant to protein denaturants (316), require a 5' free homologous end, and exhibit interwound strands. Details of this process are still unresolved, but several reports have indicated a need for SSB in this synthesis (120, 166, 316, 349, 409). SSB significantly enhances the rate of joint formation (57, 308, 316, 343). Riddles and Lehman (316), Bryant et al. (57), and Schutte and Cox (342) found a 10-fold stimulation of parane-
mic joint formation at saturating concentrations of RecA protein and SSB at 1 monomer per 8.0 nucleotides. No D-loop formation was noted in the absence of binding protein (166). In contrast, Honigberg et al. (159) reported that SSB is not essential and that suitable ionic conditions could obviate the need for SSB in the in vitro assay.

(ii) Suggested roles for SSB. Two functions have been proposed for SSB in synopsis. The SSB could bind to the displaced strand of the heteroduplex DNA in the junction area and prevent reannealing (57, 316). Alternatively, SSB may maintain stability of a RecA-ssDNA complex to ensure a high concentration for this intermediate substrate (99). Because this complex is a key intermediate linking phases 1 and 2 of the strand exchange process, it was suggested that SSB is essential to maintain filament integrity (95). Benedict and Kowalczykowski (33) used a mutant RecA protein with a truncated C terminus to look at filament integrity. The C-terminal region includes a very acidic domain believed to be involved in DNA-binding modulation. They found that the truncated protein itself actually increased the homologous pairing activity compared with wild-type RecA, but that inhibition occurred in the presence of SSB (33). Perhaps SSB moderates the binding efficiency of RecA during pairing by playing an active role as a specific assembly factor.

Recently, Chow et al. (80) reported that SSB can suppress the formation of networks which link joint molecules. This suppression inhibits the reinitiation of homologous pairing, resulting in a blockage to the reinvasion of heteroduplex DNA by the displaced strand (80). The authors suggested that the nucleoprotein filament produced in the presence of SSB remains on the heteroduplex, accelerating the forward reaction. Helix-destabilizing proteins may alleviate topological or steric barriers to the propagation of strand exchange (80). These data again suggest that SSB is essential for proper synopsis.

As Cox and Lehman (96) have pointed out, more extensive structural and kinetic analyses are required to understand the mechanistic details of the synaptic phase. The exact role of SSB in homologous pairing is not clear.

Radding (306) has also noted that direct identification of two binding sites on RecA to enable a pairing event has yet to be demonstrated. The interaction with SSB may be responsible for a conformational change in the RecA monomer to permit a fluctuation of binding sites, or, alternatively, this interaction could promote a dimerization of two RecA monomers and thereby alter a binding site. It is also possible that a monomer of SSB could dimerize with a monomer of RecA to accomplish the dual binding mode.

Strand exchange events. The third phase of in vitro recombination, strand exchange, appears to be the most complex event. At present three molecular mechanisms have been proposed to explain the events required for the DNA molecules to reach resolution (97, 301). The end products are a linear ssDNA molecule and a new heteroduplex (Fig. 9). The process is very ordered. The ssDNA of the nucleoprotein filament determines the directionality of strand exchange (94), which is 5'->3' with respect to the initiating strand (370). Because the process shows precise polarity, it is possible that the SSB protein will be needed to maintain the stability of the complex. In 1983, Bianchi and Radding (40) presented data which indicated that SSB was not essential for strand exchange. At the same time it was reported that SSB had a modest effect on the exchange process, but that it certainly increased the efficiency of ATP hydrolysis required during this stage (98). Livneh and Lehman (216) reported a requirement for SSB during strand exchange with UV-irradiated DNA; their product yield decreased by 60% without SSB. A similar decrease was also reported by others (166). Currently there is no evidence to indicate that any other protein aids RecA in this “recombinase” action (305), although it would seem likely that the physical manipulations of the DNA molecule required at this stage could involve a topoisomerase or helicase (370, 402).

SSB-RecBCD Interaction

RecBCD pathway. The RecBCD enzyme plays an active role in recombination (Fig. 10). It has three enzymatic
activities: nuclease, adenosine triphosphatase, and helicase (139, 407). In contrast to RecA, RecBCD is heterotrimERIC (8). Before 1986, RecBCD was known as RecBC or exonuclease V (425). Amundsen et al. (8) reported a new gene, recD, adjacent to the recB gene and purified the recD gene product as a 58-kDa polypeptide. It was found to be a component of and involved with the exonuclease activities of a RecBCD holoenzyme (8, 77). Surprisingly, this activity is not essential for homologous recombination, as is the helicase function (364), an activity thought to be associated with the RecB or RecC subunit (227). Although each polypeptide seems to have its own individual activity, all three are required for a fully functional recombinase enzyme (8). The in vivo activities of RecBCD include not only genetic recombination but also recovery from DNA damage and maintenance of cell viability (8).

Components required for RecBCD pathway. Models from in vitro studies propose both an early (preparation of the DNA substrate) and a late (resolution) function for RecBCD in recombination (362, 381, 407). However, it cannot complete all the enzymatic events alone. From genetics studies, a requirement for at least five other proteins in the RecBCD pathway has been demonstrated; these proteins are RecA, SSB, DNA gyrase, DNA polymerase I, and DNA ligase (361). Additional details of RecBCD can be found in several references (96, 305, 361, 362, 381). At present, we do not know with certainty the structure-function relationships of the three individual subunits. Binding studies indicate that RecBCD prefers a linear dsDNA substrate for its helicase activity (379). In contrast to the specificity seen for helix unwinding, in vitro assays indicate a broad substrate interaction for the nucleolytic function. This is especially true if no SSB is present to protect ssDNA from digestion (233). In vitro, this helicase generates two classes of intermediates: (i) duplexes with long single-stranded tails of several thousand nucleotides at the 3' and 5' ends; and (ii) ssDNA fragments of several hundred nucleotides long (233). If SSB is present in the assay, the ssDNA becomes resistant to both the endo- and exonucleolytic activities of RecBCD. Linear duplex DNA continues to be unwound. Duplex circular DNA containing short, single-stranded gaps is also resistant to hydrolysis if the binding protein is present (234). Therefore, SSB favors the ATP-dependent helicase activity of this enzyme while inhibiting its exonuclease function. For recombination to take place by this pathway, such action is needed. Other in vitro studies support a requirement for both RecBCD and SSB to generate an ssDNA substrate for the strand exchange process discussed above (section on strand exchange events). The intermediate products formed, as visualized by electron microscopy, included stable ssDNA from 5,000 to 35,000 nucleotides long (323), loop tails (377), single-stranded bubbles (282), and twin loops (282, 377). All of these intermediate products were coated with SSB (282, 377). The addition of more SSB resulted in an increase in the level of these intermediates (379). These studies illustrate the functioning of SSB in stabilization of the helix, protection of ssDNA from nucleolytic attack, and prevention of renaturation (323), i.e., functions expected for an ssDNA-binding protein. Although SSB is not directly involved in the production of ssDNA products, it is required if these structures are to remain competent.

Model for RecBCD pathway. One current model (Fig. 10) for recombination promoted by RecBCD includes SSB as well as RecA (362) and is supported by extensive genetic and enzymatic evidence. The use of purified RecBCD, RecA, and SSB in vitro verified at least the initial steps of this model. The initiation event is the binding of RecBCD to linear dsDNA at blunt free ends (379). After binding, the enzyme tracks along the DNA and begins to unwind the double strands (323). It continues its helicase activity until it encounters a Chi site, a recombogenic hot spot (298, 364, 379), of which there are 800 or more on the E. coli chromosome (405). A Chi site contains the following consensus sequence: GCTGGTGG (365). Endonucleolytic cleavage by RecBCD on just one strand occurs to six nucleotides downstream from the 3' end (78, 377). This cutting action is enhanced by SSB (78). After cleavage, helicase activity continues until the release of a 3'-OH ssDNA tail (362). It is at this point that SSB is needed. The free ssDNA end serves as the substrate for presynapsis (180) involving RecA and SSB, as discussed above (section on pre-synaptic events).

There is little, if any, evidence of the role of RecBCD during strand exchange events or after the heteroduplex junction point is formed. It has been proposed that the Chi site may promote the dissociation of the RecD subunit from the holoenzyme, converting the protein into an active recombination (381) for resolution. No data exist to indicate a need for any other protein or for an interactive complex of RecA-SSB-RecBCD leading to resolution of the heteroduplex.

ssb mutational effects. It has been noted earlier that strains carrying the ssb-1 mutation have reduced recombination (136, 141). Two experiments provide additional data for an in vivo requirement for SSB (110, 141). In one report, the foreign DNA was a derivative of phage λ containing Chi sites introduced into ssb-1 cells. A 50-fold reduction of recombination at 32°C was observed (110). Golub and Low (141) used phage λ to analyze recombination between phages or with homologous regions on the chromosome. The host chromosome contained a resident homimmune prophage to prevent replication. Prior to infection, one phage was UV irradiated to cause an indirect stimulation of genetic recombination. (This stimulation is not dependent on induction of the SOS system.) When the strain carrying the ssb-1 mutation was challenged with this dual phage system, a drastic decrease in recombination was observed (141).

Interactions of SSB in RecE and RecF Pathways

Minor pathways of recombination. The RecE and RecF pathways represent two minor pathways of homologous recombination in E. coli (87) which were observed from studies of extragenic suppressors of recBC mutations (85). These secondary pathways are revealed when recombination occurs in the absence of RecBCD (160, 178, 363).

The RecE pathway by definition is that which operates in sbcA (suppressor of recBC) mutants (361). The major protein is the product of the recE gene located within a cryptic prophage named ruc (for recombination activation), present in certain strains of E. coli K-12 (135, 169). Conjugation-associated and transduction-associated recombination events via this pathway require both recA and recF gene products, although plasmid recombination is independent of their functions (117). The mechanisms of this pathway are thought to be similar to that of the lambda Red pathway (361).

Gene products required for RecE and RecF pathways. The RecF pathway is observed if mutations are present in the sbcB and sbcC genes. The sbcB mutations inactivate exonuclease I, whereas the sbcC gene product is unknown (363). The efficiency of this system for conjugal or transductional recombination approaches that of wild-type levels (405). The
enzymes of this pathway promote the replacement of relatively long stretches of single strands of recipient DNA by the corresponding strands of donor DNA (236). The pathway promotes circular plasmid recombination in wild-type cells (117, 178, 363). Its regulation has been reported to be under the control of the LexA repressor and therefore is a part of the SOS pathway (178, 217).

The two recombination pathways are not independent, but show differences in efficiency and substrate preference (405). The initiation event for both seems to depend upon the presence of a 3′ single-stranded terminus on the DNA substrate. If this terminus is removed, the RecBCD pathway is favored (87). Furthermore, neither the RecE or RecF pathway is activated by Chi sites (363). Both pathways require other gene products, namely recA, recE, recF, recJ, recO, and recQ products. The RecF pathway also needs the recN, sbcB, sbcC, and ruv gene products (363).

To date the only proteins of these two pathways which have been isolated and purified to homogeneity are RecA, RecE, the sbcB product, and RecJ (226, 363). Consequently, most of the information has come from genetic analyses. The interactions of RecA and SSB have already been discussed above (section on SSB-RecA interaction). The remaining three proteins, RecE, the sbcB product, and RecJ, all have exonuclease activity (32, 135, 196, 226). Each has a unique substrate specificity. The gene product of recE, exonuclease VIII (32, 135), prefers linear dsDNA, hydrolyzing one strand at the 5′ end and resulting in the production of 3′ tails. Long ssDNA molecules are produced either free or attached to dsDNA (165). The sbcB gene encodes exonuclease I (196), with a 3′-5′ specificity on ssDNA (207). The loss of exonuclease I activity alone cannot restore the recombination proficiency of E. coli cells; an additional mutation at the sbcC locus is required (362). The presence of exonuclease I (i.e., sbcB+) causes inhibition of recJ-dependent recombination in certain strains (225). The most recent gene product identified, that of the recJ gene (160), requires a free single-stranded end on a linear DNA substrate (226). It does not hydrolyze linear dsDNA and has no endonuclease activity. Degradation appears to be preferential at the 5′ end (226). RecJ may be able to substitute for one or more of the normal activities of the RecD subunit of the RecBCD holoenzyme (227). Each of these three nucleases (exonuclease VIII, exonuclease I, and RecJ nuclease) can produce ssDNA tails which may be bound by SSB in the preparation of the DNA for RecA-dependent strand exchange (407).

Possible roles of SSB in minor pathways of recombination. There is little information at present on possible interactions of SSB with the other proteins of these minor pathways. RecF has not yet been purified to homogeneity (235), even though the gene was identified in 1973 (160). Physiological studies of mutant strains have implicated roles for this protein in regulation of the SOS response (393), UV mutagenesis (84), DNA repair (217), and gene conversion (116). From a combined in vivo study of recombination in a recF recA mutant strain and an in vitro assay with the purified RecA mutant protein, an interaction of RecA, RecF, and SSB has been suggested (235): (i) RecF might counteract inhibition by SSB during the binding competition for ssDNA early in the strand exchange process; (ii) RecF might be involved in the complex formation of the nucleoprotein filament intermediate; or (iii) RecF might modify the ssDNA substrate so that SSB would be unable to inhibit RecA binding (235). The possibility that RecF assists RecA in binding to ssDNA was first suggested by Blanar et al. (41) and is supported by later work (277). It has also been suggested that RecF may help RecA to release SSB from ssDNA (277).

As pointed out by Clark et al. (87), the full importance of SSB for recombination in the various genetic backgrounds has not been adequately assessed. The limited information on the minor pathways of recombination in E. coli does not allow an assessment of other possible roles for SSB protein at present.

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