Biochemical and biological function of *Escherichia coli* RecA protein: behavior of mutant RecA proteins

SC Kowalczykowski

Department of Cell, Molecular, and Structural Biology, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, IL 60611, USA

Summary — The recA protein of *E coli* participates in several diverse biological processes and promotes a variety of complex *in vitro* reactions. A careful comparison of the phenotypic behavior of *E coli recA* mutations to the biochemical properties of the corresponding mutant proteins reveals a close parallel both between recombination phenotype and DNA strand exchange and renaturation activities, and between inducible phenomena and repressor cleavage activity. The biochemical alterations manifest by the mutant recA proteins are reflected in the strength of their interaction with ssDNA. The defective mutant recA proteins fail to properly assume the high-affinity DNA-binding state that is characteristic of the wild-type protein and, consequently, form less stable complexes with DNA. The mutant proteins displaying an 'enhanced' activity bind ssDNA with approximately the same affinity as the wild-type protein but, due to altered protein–protein interactions, they associate more rapidly with ssDNA. These changes proportionately affect the ability of *recA* protein to compete with SSB protein, to interact with dsDNA, and, perhaps, to bind repressor proteins. In turn, the DNA strand exchange, DNA renaturation, and repressor cleavage activities mirror these modifications.

genetic recombination / DNA strand exchange / recA protein / SSB protein / repressor cleavage

Introduction

The recA protein of *Escherichia coli* is essential for both homologous recombination and inducible responses to DNA damage. Its participation in these processes requires 2 remarkably different biochemical activities; its ability to promote homologous pairing and DNA heteroduplex formation is central to models for genetic recombination [1, 2] while its ability to cleave the *lexA* and phage repressors and the umuD protein is central to inducible phenomena [3, 4].

In vitro, recA protein promotes both the renaturation of single-stranded DNA (ssDNA) and the exchange of DNA strands between a variety of complementary DNA substrates [5–7]. DNA strand exchange has 3 requirements: a region of ssDNA complementary to its homologue, a free DNA end to relieve topological constraints, and the formation of an active complex of NTP-recA protein ssDNA. This complex plays a central role in all recA protein-dependent activities and is the active species for ATP hydrolysis, repressor cleavage, and homologous pairing.

DNA strand exchange between circular ssDNA and linear dsDNA is one of the most extensively studied reactions promoted by recA protein (fig 1). The reaction is a multi-step process characterized by a number of kinetically distinct steps. The first is the binding of recA protein to ssDNA, resulting in the formation of a protein saturated (1 recA protein monomer per ~ 3 nucleotides) presynaptic complex [11]. The presynaptic complex is active in ATP hydrolysis, and its formation is normally stimulated by SSB protein or by conditions that destabilize ssDNA secondary structure [12–14]. The active presynaptic complex can promote formation of non-homologously paired networks with dsDNA, referred to as coaggregates [15]. Upon recognition of complementary contacts, homologously paired structures called joint molecules form. Following formation of the nascent heteroduplex joint, recA protein-promoted DNA heteroduplex extention occurs in a 5' to 3' direction relative to the displaced, non-complementary strand [16]. The ultimate reaction products are nicked or gapped circular dsDNA and linear ssDNA, provided that re-invasion by the displaced strand does not occur [17, 18]. Normally, ATP hydrolysis occurs during DNA strand exchange, but it is not essential for formation of up to 3.4 kb of heteroduplex DNA [19].
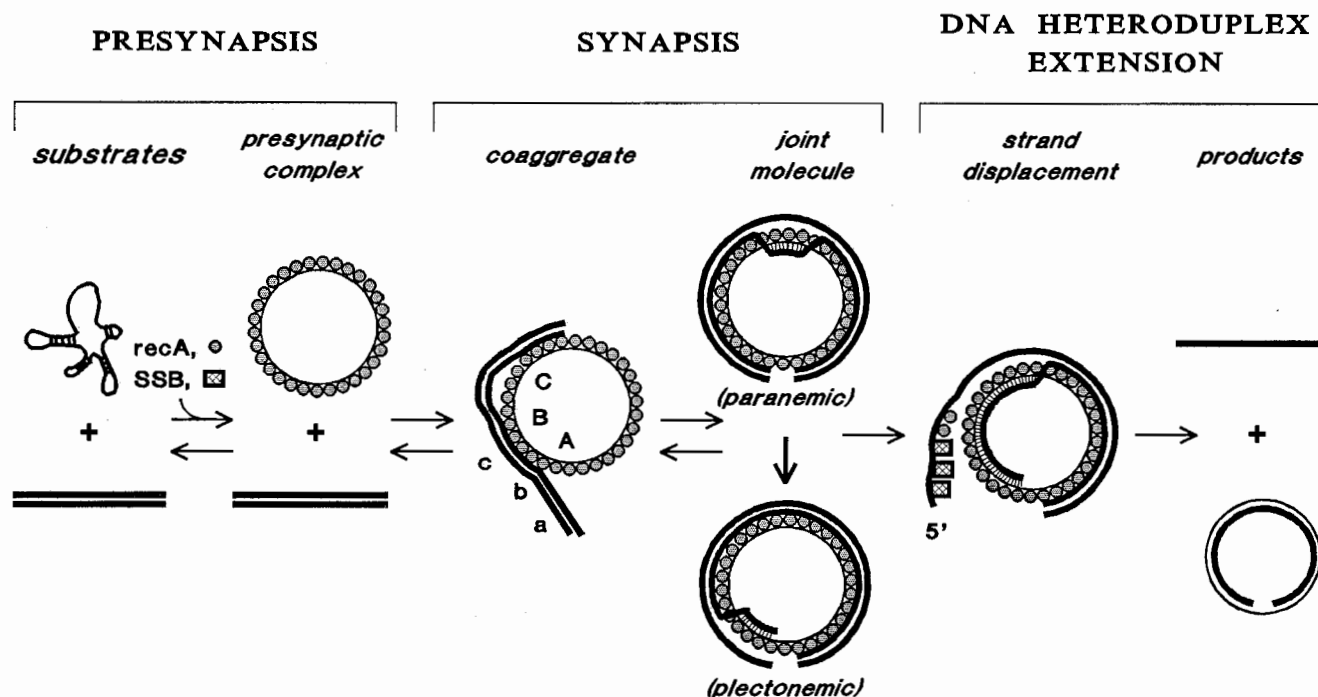
Fig 1. A model for recA protein promoted DNA strand exchange (adapted from [10]).

The repressor cleavage activity of recA protein is also quite distinctive. While the active form is a ternary complex of recA protein, ssDNA and ATP, ATP hydrolysis is not essential since ATP analogs such as ATPγS and ADP•AlF$_4^-$ can effect repressor cleavage [20, 21]. The cleavage reaction promoted by recA protein is unlike that of conventional proteases in that the repressor protein is capable of auto-digestion in the absence of recA protein [22]. Thus, recA protein appears not to participate directly in the chemistry of the cleavage reaction but rather enhances the auto-digestion rate by binding to the substrate protein [109].

Given the complexity and diversity of the *in vitro* reactions promoted by recA protein, it is fair to ask which of these reactions are essential for cellular function. This question can be answered by a careful comparison of the phenotypic behavior of *recA* alleles with the biochemical behavior of the purified mutant protein. In addition to providing insight into biological issues, the properties of mutant recA proteins should help clarify some of the mechanistic issues surrounding the *in vitro* DNA strand exchange reaction as well as provide information regarding structure–function relationships. Given these considerations, the biochemical properties of mutant recA proteins are reviewed; detailed discussion is restricted to only those proteins for which there is both genetic and biochemical information.

Recombination functions

DNA strand exchange activity correlates with in vivo *recombination function*

The ability to promote the exchange of DNA strands is an activity unique to recA protein (and recA-like proteins) [23] and, consequently, is certain to reflect an activity crucial to genetic recombination. This presumption is substantiated by the biochemical behavior of the mutant recA proteins that have been purified to date (table I). The correlation is exact: those mutations having a null phenotype produce a protein without DNA strand exchange activity (recA1, recA13, recA56, and recA142 proteins); that having reduced recombination function displays reduced DNA strand exchange activity (recA430 protein); and those having enhanced recombination function display enhanced DNA strand exchange activity (recA441 and recA803 proteins). This correlation holds true for the reaction involving recA protein alone as well as for an *in vitro* reaction requiring the concerted action of recA, recBCD, and SSB proteins [24].

For the alleles that are defective in genetic recombination, the respective proteins fail to promote any homologous pairing or DNA strand exchange under all conditions examined (*ie* in the presence or the absence of SSB protein, at various magnesium ion

Table I. Properties of *E coli* mutant RecA proteins

	1202	1211	730	803	441ᵃ	wt	629	430ᵇ	142	1	56	13
In vivo properties:												
recombination[c]	+	+	++	++	++	+	+/–[d]	+/–	--	--	–	–
SOS induction[e]	++	++	++	+	++	+	nd[f]	+/–	+/–	–	–	nd
prophage induction[g]	++	++	++	nd	++	+	+	–	+/–	–	nd	nd
In vitro properties[h]:												
DNA strand exchange[i]	nd	nd	++	++	++	+	nd	+/–	–	–	–	–
DNA renaturation[j]	nd	nd	nd	nd	+/– (.7)	+ (1)	+/–[k]	+/–(.4)	– (0)	– (0)[l]	nd	nd
ssDNA binding[m]:												
no cofactor	nd	nd	+ (1)	+/– (.9)	+/– (.6)	+ (1)	nd	+ (1)	+ (1)	+/– (.9)	+/– (.6)	+/– (.2)
with ADP	nd	nd	++ (1.1)	+/– (.9)	+ (1)	+ (1)	nd	+(1)	+ (1)	nd	+/– (.7)	+/– (.3)
with ATP	nd	nd	+/– (.8)	+/– (.6)	+/– (.8)	+ (1)	nd	+/– (,8)	+/– (.3)	+/– (.2)	+/– (.1)	– (0)
RFI with ATP[n]	nd	nd	+	+	+	+	nd	+	–	–	–	–
association rate[o]	nd	nd	nd	++	++	+	nd	+/–	nd	nd	nd	nd
ssDNA ATPase[p]:												
without SSB[q]	++[r]	++[s]	++	++	+	+	+/–[t]	+	+[u]	+/–[v]	–	–
K_m (μM)[w]	19	23	nd	nd	23	60	nd	85	400	> 300[x]	none	none
with SSB[y]	nd	nd	++	++	++	+	nd	+/–	–	–	--	–
SSB displacement[z]	nd	nd	+++	++	++	+	nd	+/–	--	–	–	–
dsDNA ATPase[aa]	nd	nd	nd	++	+	+	nd	+/–	+/–	–	–	nd
Coaggregation[ab]	nd	nd	nd	nd	+	+	nd	+/–	–	–	–	nd
lexA cleavage[ac]:												
without SSB	++	++	nd	nd	++	+	nd	+/–	+/–	nd	--	nd
with SSB	nd	nd	nd	++	++	+	nd	+/–[ad]	--	nd	--	nd
λ repressor cleavage[ae]	nd	nd	nd	nd	++	+[af]	+	--	+/–	–	nd	nd

The data in this table is compiled from the following references: *recA1* [20, 25, 26, 39, 50, 97] SD Lauder and SC Kowalczykowski, unpublished results; *recA13* [98], SD Lauder and SC Kowalczykowski, unpublished results; *recA56* [99–101], SD Lauder and SC Kowalczykowski, unpublished results; *recA142* [27, 28, 40, 52, 59, 102, 103]; *recA430* [25, 30, 40, 52, 59, 66, 104, 105]; *recA441* [18, 31, 40, 45, 53, 66, 106]; *recA629* [34a, 64, 94]; *recA730* [32], PE Lavery and SC Kowalczykowski, unpublished results; *recA803* [34, 46] PE Lavery and SC Kowalczykowski, unpublished results; *recA1202* [62, 63, 92]; *recA1211* [62, 63, 92]; *recAwt* [14, 20, 44, 53, 55, 107, 108].

ᵃThe entries for recA441 protein are for 42°C and in the absence of adenine. ᵇThe biochemical properties are unusally sensitive to protein concentration and the type of nucleotide cofactor present; the entries below are for typical conditions but, where significant, the behavior in the presence of dATP is noted. ᶜBased on either conjugal recombination, P1 transduction, or host-dependent lambda recombination. ᵈRecombination function is cold-sensitive, being defective at 30°C. ᵉExpression of genes regulated by *lexA* repressor. ᶠNot determined. ᵍInduction of bacteriophage lambda. ʰBecause the biochemical properties of recA protein are dependent on reaction conditions, the entries in this table are for commonly used reaction conditions; these conditions are typically Tris buffer pH 7.5, 8–12 mM (Mg⁺²), 0.5–1 mM ATP, and no added salt at 37°C. Where important, variations from these typical conditions are noted. ⁱDNA strand exchange activity between using circular ssDNA and linear dsDNA in the presence of SSB protein as measured by the agarose gel assay; where examined, similar results are obtained using other assays and DNA substrates. ʲATP-dependent renaturation of either alkali- or heat-denatured ssDNA using a 30:1 ratio of nucleotide to recA protein. ᵏATP-dependent renaturation is ~3-fold reduced at 28°C, compared to wild-type protein; ATP-independent activity is unaltered. ˡRecA1 protein is fully proficient in ATP-independent renaturation. ᵐBinding to etheno M13 DNA in the presence of the nucleotide indicated. The values in parentheses represent the salt concentration of NaCl required to dissociate the complex (STMP) relative to that required to dissociate the wild-type protein complex; these ratios are not a ratio of DNA binding affinities because the relationship between STMP and affinity is logarithmic. Typical STMP values (in mM NaCl) for wild-type recA protein are: 230–300 in the absence of nucleotide cofactor; 140–160 in the presence of 250–500 μM; and 600 to 900 mM in the presence of 500 μM ATP. ⁿRelative fluorescence increase in the presence of ATP; a '+' indicates the higher value typical of the high affinity state, '–' indicates the lower value. ᵒRate of association with etheno M13 DNA in the presence of ATP at 150–200 mM NaCl. ᵖActivity is measured using either M13 ssDNA or heat denatured lambda DNA. �ۧRefers to the observed rate of hydrolysis. A '++' indicates that ssDNA normally unavailable to wild-type is being utilized to support ATP hydrolysis. ʳThe designation '++' is due to a Km for ssDNA that is 3-fold lower than the wild-type value. ˢThe designation '++' is due to a Km for ssDNA that is 2-fold lower than the wild-type value. ᵗATPase activity is reduced ~3-fold at 28°C compared to wild-type protein. ᵘThe activity is more salt-sensitive than that of wild-type. ᵛA nearly wild-type level of ATP hydrolysis is detected at pH 6.2. ʷThe ATP concentration-dependence of ATP hydrolysis is sigmoid; the value reported is the ATP concentration at one-half of full activity (S₀.₅). ˣThe S₀.₅ at pH 6.2 is 45 μM. ʸSaturating SSB protein added after recA protein. ᶻSSB protein displacement measured as the relative time required to achieve steady-state ATP hydrolysis after addition of recA protein to saturated complexes of SSB protein and M13 ssDNA; times faster than wild-type protein are indicated by '++' or '+++'. ᵃᵃMeasurements of the lag time determined using M13 linear dsDNA; a shorter lag time than wild-type protein is indicated by a '++'. ᵃᵇAbility to pellet ssDNA and non-homologous dsDNA by centrifugation. ᵃᶜIn the presence of ssDNA and ATP; when SSB is present, it is added after formation of the recA protein-ssDNA complex (SSB-second protocol). ᵃᵈThere is no detectable cleavage in the presence of SSB protein when ATP is the cofactor; however, when dATP is the cofactor, the cleavage rate is ~50% of the wild-type rate. ᵃᵉIn the presence of ssDNA and ATP; SSB protein is absent. ᵃᶠATP-γ-S-dependent cleavage is inhibited in the presence of excess SSB protein.

concentrations, and at various concentrations of ATP or dATP [25–28], SD Lauder and SC Kowalczykowski, unpublished results). The only exception is recA142 protein, which can promote joint molecule formation in the presence of ATPγS (SC Kowalczykowski and RA Krupp, unpublished results). ATPγS is a potent allosteric inducer of the high-affinity DNA binding state of recA protein [10] which can induce this conformation of recA142 protein whereas ATP does not. Though reactions in the presence of ATPγS are not applicable to the physiological situation, they provide information relevant to biochemical mechanism.

The recA430 mutation reduces conjugal recombination function to 40% of the wild-type level [29]. In vitro, recA430 protein has reduced DNA strand exchange and displays a sensitivity to both protein concentration and NTP used [25, 30]. In the presence of ATP, the rate of joint molecule formation is 9-fold lower than for wild-type protein and no final product is detected; however, in the presence of dATP (which is a better effector of the high-affinity state [10]), the rate of joint molecule formation is 6-fold lower than wild-type protein and product formation (ie DNA heteroduplex extension) is reduced only 2.5-fold. Finally, unlike wild-type protein, increasing the recA430 protein concentration by 2-fold can increase the rate of joint molecule formation by 14-fold.

In contrast to the defective recA alleles, the recA441 mutation causes an increased frequency of genetic exchanges per unit length of chromosome [31], the recA730 mutation increased recombination in one-point transductions [32], while the recA803 mutation suppresses the recombinational defect of recF mutations [33]. Thus these mutations result in recA proteins with 'enhanced' activity relative to wild-type protein. In agreement with the physiological observations, the rates of joint molecule formation and DNA strand exchange by recA441 protein are greater than those of wild-type protein when examined under sub-optimal reaction conditions [18]. This enhancement is most prominent when the ssDNA is complexed with SSB protein, requiring displacement of SSB protein by recA protein. In this situation, joint molecules formation by recA441 protein is 5-fold faster than wild-type protein. In addition, due to the increased ability of recA441 protein to dislodge SSB protein from the displaced DNA strand, reinvasion by this strand occurs, leading to homology-dependent network formation. Like recA441 protein, recA803 protein can promote joint molecule formation more than 10-fold more rapidly than wild-type protein when the ssDNA is pre-complexed with SSB protein [34]; unlike recA441 protein, recA803 protein can utilize ssDNA that is otherwise sequestered in stable contrary structure, resulting in a 3-fold faster rate of joint molecule formation in the absence of SSB protein.

These observations suggest that the suppression of recF mutations by recA803 protein results from these enhanced activities and implies that the recF protein normally provides such functionality to the wild-type recA protein. Finally, the recA730 protein manifests both of the enhancements exhibited by recA803 protein (PE Lavery and SC Kowalczykowski, unpublished results). Whether this recA allele can suppress recF mutations or exhibits enhanced recombination function is unknown. Thus, in vitro DNA strand exchange activity correlates with in vivo recombination function without exception.

DNA renaturation activity correlates with in vivo recombination function

Both the uniqueness of the DNA strand invasion reaction and its prominence in models for genetic recombination make this activity the logical candidate for the essential in vivo function of recA protein; however, its DNA renaturation activity cannot be overlooked [34a]. Though renaturation activity may reflect a partial activity of DNA strand exchange activity, it may also represent a physiologically important function of recA protein. Many ssDNA binding proteins catalyze the renaturation of ssDNA [35] but the reaction characteristics differ from renaturation promoted by recA protein [36–38]. Renaturation catalyzed by SSB protein and T4-coded gene 32 protein requires sufficient protein to saturate the ssDNA and is second order in ssDNA concentration; removal of secondary structure by these proteins is the primary mechanisms responsible for accelerated renaturation. In contrast, recA protein-promoted renaturation is optimal at sub-saturating protein concentrations (1 protein per 30 nt), is first order in ssDNA concentration, and is stimulated by ATP hydrolysis; thus, recA protein accelerates the reaction rate by binding different ssDNA molecules in a ternary complex and holding them in proximity until pairing and renaturation occur.

Though the renaturation activity of mutant recA proteins has not been examined in detail, reasonable agreement with recombination phenotype exists. RecA1 and recA142 proteins essentially promote no renaturation in vitro [39, 40]; and recA441 displays 70% of wild-type activity [40]. The trends observed for renaturation of ssDNA by these mutant proteins are also paralleled in an in vitro reaction requiring both the helicase activity of recBCD enzyme and the renaturation activity of the recA protein [40]. The recA629 protein is particularly interesting because its ATP-dependent renaturation activity (but not its ATP-independent activity) is cold-labile, in agreement with its cold-sensitive recombination-deficient phenotype [34a]. Since recA protein-promoted renaturation of ssDNA is inhibited by SSB protein [37], for this

activity to have a cellular function, it can only be manifest under physiological conditions where the free SSB protein is titrated by formation of (excess) ssDNA. Such conditions may exist upon UV irradiation or introduction of other DNA damage which produce ssDNA through either stalled replication or DNA degradation. Since SSB protein is not significantly induced by DNA damage [41, 42], the production of only ~100 kb of ssDNA is sufficient to titrate all of the SSB protein (assuming ≈ 7000 monomers per cell [43]) and to alleviate the inhibition of recA protein renaturation activity. This amount of ssDNA is equivalent to that produced by the unwinding/degradation of just one lambda DNA molecule. Thus, recA protein-promoted renaturation may be involved in the repair of extensively damaged DNA.

Altered single-stranded DNA binding properties underlie the biochemical modifications

The first step in DNA strand exchange, DNA renaturation, and repressor cleavage is the binding of recA protein to ssDNA. There are at least 2 types of complexes: 1), those that form in the presence of ATP or ATPγS, resulting in the induction of the high-affinity DNA binding state of recA protein; and 2), those that form in the presence of ADP or in the absence of cofactor, resulting in the induction of the low-affinity DNA binding state [10]. As the terms imply, the high-affinity state has a high affinity for DNA and is characterized by the extended structure visible by electron microscopy; the low-affinity state has a low affinity for DNA and a more compact structure. The formation of either state is readily quantified using the fluorescent ssDNA referred to as etheno M13 DNA [44]. A measure of ssDNA binding affinity is the NaCl concentration required to dissociate one-half of the recA protein-DNA complex and is termed the salt titration midpoint (STMP). A second characteristic is the fluorescent enhancement upon complex formation (referred to as the relative fluorescence increase or RFI), which is higher for the high-affinity state than for the low-affinity state.

The failure of recA1, recA13, recA56, and recA142 proteins to promote DNA strand exchange is not due to an inability to bind ssDNA (table I) [25–28] (SD Lauder and SC Kowalczykowski, unpublished results). In the absence of nucleotide cofactor, these proteins bind etheno M13 DNA with the same stoichiometry and with nearly the same affinity as wild-type protein. However, in contrast to wild-type protein, the addition of ATP to complexes with any of the first 3 proteins results in complexes that dissociate at low salt concentrations, demonstrating that ATP binding results in a complex with a lower affinity for ssDNA. In addition, the RFI is low, which signifies

formation of the compact rather than extended conformation.

The ssDNA binding properties of recA142 protein are more complex [27]. Elevated concentrations of ATP induce a conformation with a stability that is higher than that of the nucleotide-free protein, but less stable than that of the wild-type protein ATP–complex. In addition, ATP binding does not induce the high RFI although ATPγS binding does. This observation is consistent with the requirement of the high affinity state for DNA strand exchange activity, since recA142 protein is unable to promote DNA strand exchange in the presence of ATP but is able to do so in the presence of ATPγS.

The recA430 protein also exhibits altered DNA binding behavior [25, 30]. The STMP values for both the ATP- and the dATP-bound species of recA430 protein are 25–35% lower than the corresponding wild-type complex. However, in contrast to recA142 protein, both nucleoside triphosphates induce the high RFI form. These observations are consistent with the capacity, albeit reduced, of recA430 protein to promote DNA strand exchange.

If the trend exhibited by the defective mutant recA proteins were extrapolated to the 'enhanced' mutant proteins, then recA441, recA730, and recA803 proteins might bind ssDNA more tightly than the wild-type protein. This, however, is not the case ([45, 46], PE Lavery and SC Kowalczykowski, unpublished results). In the presence of ATP, these proteins bind etheno M13 DNA with an apparent affinity that is equal to or somewhat less than that of the wild-type protein. Thus, the explanation for their enhanced DNA strand exchange activity cannot be found in the stability of their protein–ssDNA complexes. Instead, the only enhanced ssDNA binding property found for recA441 and recA803 proteins is the rate at which they associate with ssDNA. This assembly process is a nucleation-limited reaction whose molecular nature is unknown [47], but 2 possibilities exist. Nucleation may require either the assembly of a critical-sized nucleus prior to ssDNA binding or, alternatively, the disassembly of a non-productive protein aggregate that inhibits binding.

In summary, the ssDNA binding properties of the mutant recA protein demonstrate a need for induction of the high-affinity state. However, though necessary, this state is not sufficient nor can it explain the enhanced activity of the recA441 and recA803 proteins. For these 2 proteins, an increased rate of association with ssDNA appears to provide an explanation.

Competition with SSB protein parallels the ssDNA binding properties

The binding of recA protein to ssDNA is only one aspect of presynaptic complex formation. Normally,

presynaptic complex formation results in ATP hydrolysis. Although ATP hydrolysis is neither necessary nor sufficient for DNA heteroduplex formation, ATP hydrolysis serves as a useful indicator of the formation of the ATP-recA protein–ssDNA complex. SSB protein stimulates presynaptic complex formation by removing DNA secondary structure which limits recA protein binding [13, 14, 48] (fig 2). The stimulatory effect of SSB protein can be partially mimicked by preincubating the presynaptic complex in low (1 mM) magnesium ion concentration, but due to recA protein turnover, this transient effect decays upon transfer of the complex to the higher magnesium ion concentrations (> 6 mM) that are conducive to DNA strand exchange [13, 14, 49]. In contrast to the

stimulatory effects, SSB protein can also inhibit recA protein-dependent activities [14, 27, 28, 30, 45, 50, 51]. Under these conditions, SSB protein displaces recA protein from the ssDNA [48], thereby blocking formation of the essential recA protein-ssDNA complex.

Three of the defective mutant recA proteins (recA1, recA13, and recA56) have little or no ATPase activity [25, 26], (SD Lauder and SC Kowalczykowski, unpublished results). The recA1 protein has ssDNA-dependent ATPase activity, but only at pH 6.5; this activity is inhibited by SSB protein, which is indicative of recA protein displacement [50]. On the other hand, recA142 protein has almost normal levels of ssDNA-dependent ATPase activity under typical
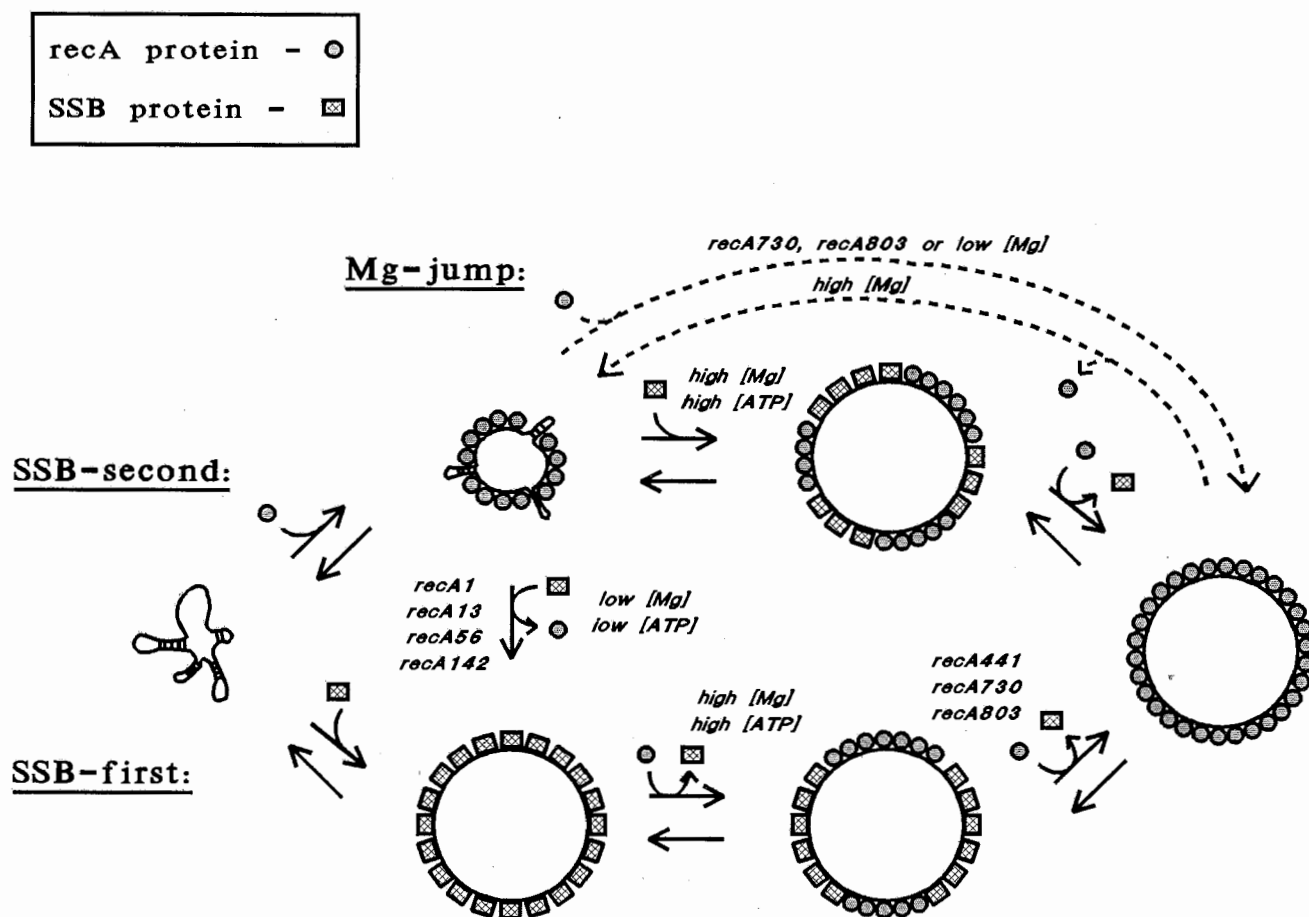

Fig 2. Competition model for the interaction of recA and SSB proteins. The pathway followed when recA protein is allowed to bind ssDNA prior to the addition of SSB protein is illustrated by the 'SSB-second' path. The pathway followed when SSB protein is allowed to bind ssDNA prior to recA protein is illustrated by the 'SSB-first' path. The need for SSB protein is circumvented by allowing recA protein to bind ssDNA at low magnesium ion concentration, followed by an increase in the magnesium ion concentration to one amenable to DNA strand exchange (dashed lines); the resultant recA protein complexes are unstable and dissociate as shown by the return path. The altered behavior of each mutant recA protein with regard to competition with SSB protein for ssDNA binding is indicated, adapted from [10].

DNA strand exchange conditions [27, 28, 52]. This activity is more sensitive to increasing NaCl concentrations than wild-type protein [27, 52], confirming the presence of the underlying ssDNA binding defect described above. However, despite the near normal ATPase activity at low salt concentration, SSB protein completely inhibits ATP hydrolysis by recA142 protein under all conditions. Thus, consistent with the failure of recA142 protein to assume the properties of the high-affinity DNA binding state, SSB protein displaces it completely from ssDNA. An engineered variant of the recA1 mutation containing an asparagine residue instead of the aspartic acid residue also displays ATPase activity that is inhibited by SSB protein [51]. However, this inhibition is pH-sensitive and, below pH 6.7, an SSB-resistant form is formed that is proficient in DNA strand exchange; there is a direct correlation between the pH-dependent appearance of the SSB-resistant form and the capacity to promote DNA strand exchange. Finally, though the effects of SSB protein on the ATPase activity of recA629 protein have not been examined, the ssDNA-dependent ATPase is cold-sensitive, which accounts for its cold-sensitive ATP-dependent renaturation activity [34a].

In the absence of SSB protein, recA430 protein displays ssDNA-dependent ATPase and dATPase activity that is virtually indistinguishable from that of wild-type protein [30]. However, when SSB protein is present, the same sensitivities to reaction conditions that are observed in the DNA strand exchange reaction are also observed for NTP hydrolysis. Thus, ATPase activity in the presence of SSB protein is dependent on high concentration of recA430 protein, ATP, and magnesium ions; dATPase activity, in comparison, is less sensitive to these reaction variables. This close correlation suggests that most of the DNA strand exchange defect is a consequence of impaired presynaptic filament formation which results from a reduced ability to compete with SSB protein for the limited ssDNA binding sites.

The recA441, recA730, and recA803 proteins are not defective in ATPase activity; in fact, under conditions sub-optimal for wild-type recA protein activity, ATP hydrolysis by these mutant proteins is enhanced ([45, 46], PE Lavery and SC Kowalczykowski, unpublished results). One such condition is in the presence of SSB protein. At either low magnesium ion, low ATP, or low recA protein concentrations, SSB protein inhibits the ATPase activity of wild-type recA protein but not that of these mutant proteins. The most notable of the sub-optimal conditions is when SSB protein is bound to ssDNA prior to addition of recA protein (the so-called 'SSB-first' protocol; fig 2). When presented with a preformed SSB protein-ssDNA complex, wild-type recA protein displays a lag in both ATPase and DNA strand exchange activities that corresponds to the rate-limiting displacement of SSB protein from ssDNA. In addition to the lag, the steady-state ATPase activity of wild-type recA protein is less than that obtained when SSB protein is added after recA protein (the 'SSB-second' protocol; fig 2), suggesting incomplete displacement of SSB protein. In SSB-first assays, recA441, recA730, and recA803 proteins have an increased capacity to displace SSB protein as measured by both the rate and extent of SSB protein displacement. RecA441 protein can displace SSB protein at least 2-fold faster than wild-type protein; recA803 is ≈ 4-fold faster than wild-type protein and recA730 is still faster. RecA441 and recA803 proteins associate with ssDNA more rapidly than wild-type protein, and this physical property must be responsible for the increased rate of SSB protein displacement from ssDNA. (Relevant to this issue, the association rate for recA430 protein is lower than that for wild-type protein, particularly in the presence of ATP and less so in the presence of dATP [30].) A distinguishing feature of the SSB protein displacement behavior of recA441 protein is that it is temperature-sensitive; both the rate and the extent of SSB protein displacement increase more rapidly with temperature than those of wild-type protein [45]. This property may contribute to the temperature-sensitive induction of the SOS response which characterizes the *recA441* allele [54].

RecA730 and recA803 proteins exhibit a unique aspect of ATP hydrolysis. In contrast to all other recA proteins, these 2 proteins can utilize nearly all of the ssDNA that is sequestered in secondary structure and that is normally unavailable to wild-type protein as a cofactor for ATP hydrolysis ([46], PE Lavery and SC Kowalczykowski, unpublished results). This unique attribute of recA803 protein is possibly responsible for its ability to suppress the recombination defect resulting from *recF* mutations [46].

To summarize, although some mutant recA proteins show relatively unaltered ssDNA-dependent ATPase activity, distinct differences become evident in the presence of SSB protein. In fact, only in the presence of SSB protein does a direct correlation with DNA strand exchange activity become evident: the mutant proteins that compete poorly with SSB protein similarly display reduced DNA strand exchange activity; mutant recA proteins that display enhanced displacement of SSB protein are proportionately more proficient in DNA strand exchange under suboptimal reaction conditions.

Interactions with dsDNA

After formation of the presynaptic complex, the recA protein-ssDNA filament must productively interact with dsDNA. The physical basis for this interaction is

not well understood, but a number of qualitative assays exist. RecA protein can bring together ssDNA and dsDNA non-homologously in a pelletable complex called a coaggregate (fig 1), which enhances homologous pairing under certain conditions [15]. Another assay measures dsDNA-dependent ATPase activity [55]. This activity reflects the binding of recA protein to dsDNA and its topological unwinding, resulting in a complex proficient in ATP hydrolysis [55, 56]. Though the ATP hydrolysis is not necessary for homologous pairing [19], dsDNA-dependent ATPase activity presumably reflects the ability of recA protein to bind to and alter dsDNA structure, making it receptive for homologous pairing.

RecA1, recA13, and recA56 proteins are incapable of either coaggregation or dsDNA-dependent ATPase activity (SD Lauder and SC Kowalczykowski, unpublished results). Similarly, recA142 protein cannot coaggregate DNA although it does possess reduced dsDNA-dependent ATPase activity (5% of wild-type activity using M13 dsDNA) [27, 28]. The failure of recA142 protein to promote joint molecule formation in the absence of SSB protein is probably a consequence of the attenuated interaction with homologous M13 dsDNA and not of the defective coaggregation activity, because coaggregation is not essential for homologous pairing.

RecA441 and recA803 proteins possess enhanced dsDNA-dependent ATPase activity, consistent with their other increased activities [45, 46]. RecA803 protein displays a shorter lag than wild-type protein prior to steady-state ATP hydrolysis and achieves a higher final rate; for recA441 protein, these enhancements are magnesium ion- and temperature-dependent. This increased activity is probably a consequence of the increased rate of association exhibited by both of these proteins. As expected, recA441 protein forms coaggregates [48].

Inducible functions

Another essential function of recA protein is the induction of SOS response, prophage, and mutagenesis *via* cleavage of lexA, lambda cI and cp80 repressors, and umuD proteins, respectively [4]. Activation of wild-type recA protein coprotease activity requires DNA damage. Since cleavage *in vitro* requires a ternary complex of ATP, recA protein, and ssDNA, alteration of any of these interactions will affect repressor cleavage as well as recombination function. Cleavage activity is also sensitive to the presence of SSB protein because SSB protein affects formation of this ternary complex [57]. Finally, in addition to these essential interactions, recA protein must specifically bind each target protein. Thus, mutations that alter ternary complex formation will uniformly affect each

in vitro cleavage reaction, whereas mutations that alter repressor protein binding will differentially alter protein cleavage reactions.

Where examined, the mutant recA protein displaying a null phenotype fails to cleave any of the protein substrates. Purified recA1 protein cannot cleave lambda repressor protein [20] cp80 repressor protein [58], recA56 protein cannot cleave *lexA* repressor protein (SD Lauder and SC Kowalczykowski, unpublished results), and recA13 protein cannot cleave lexA protein when assayed directly in *E coli* [59].

The results obtained with the attenuated *recA* mutations, *recA430* and *recA142*, are more complex. If induced by UV-irradiation, both of these mutations result in a cleavage rate that is $\approx$ 5-fold slower than wild-type when lexA repressor cleavage is measured directly in *E coli*; if induction is by way of treatment with bleomycin, then *recA430* is completely defective and *recA142* is reduced by about one-half [59]. Consistent with these results, purified recA430 protein cleaves purified lexA protein *in vitro* 5- to 3-fold slower than wild-type protein, depending on whether rATP or dATP, respectively, is the nucleotide cofactor and provided that SSB protein is absent [30]. However, if SSB protein is present, then either no cleavage or 4-fold slower cleavage results depending on whether rATP or dATP, respectively, is the nucleotide cofactor. Purified recA142 protein also displays conditional cleavage activity: in the absence of SSB protein, lexA repressor cleavage is only 20% of the wild-type rate; in the presence of SSB protein, lexA repressor cleavage in undectable [27]. These 2 mutant alleles are also defective in prophage induction (table I); in agreement, recA430 protein fails to cleave lambda repressor protein (even in the presence of ATPγs) and recA142 protein displays only ~10% of wild-type activity [52]. Addition, *recA430* strains are non-mutable [60] and, in agreement, recA430 protein fails to cleave umuD protein *in vitro* [61].

The protease-constitutive class of mutant *recA* alleles (*recA1211, recA1202, recA730*, and *recA441*) displays induction without the need for DNA damage, yet all mutant proteins examined still require nucleic acid and NTP as cofactors for the cleavage reactions [62, 63]. The most extensively studied of these mutations is *recA441* (*tif1*). *In vitro*, recA441 protein cleaves lambda repressor protein 2- to 5-fold faster than wild-type recA protein, depending on the nucleotide cofactor present [53]. It also has a higher apparent affinity (lower K_m) for NTP [53] and can better utilize both oligonucleotides (12- to 16-mers) [53] and RNA (poly rU and poly rC) [64] as cofactors for lambda repressor cleavage. The intragenic suppressor of *tif1*, the *recA629* mutation, results in a protein that can no longer utilize RNA as cofactor for lambda repressor cleavage [64]. RecA441 protein cleaves *lexA* repres-

sor protein ~2-fold faster than wild-type protein when SSB protein is absent [45]. However, in the presence of SSB protein, this difference is amplified; this difference is particularly pronounced when displacement of DNA-bound SSB protein is required (the SSB-first protocol; fig 2). Finally, a complete explanation of the *tif* phenotype requires a rationale for the temperature-dependent SOS induction. The displacement of SSB protein from ssDNA by recA441 protein provides one explanation for this behavior, but recA441 protein also shows a slight temperature-dependent ability to use dsDNA as a substrate for *lexA* repressor cleavage [45]. Finally, consistent with these enhanced cleavage activities, recA441 protein cleaves umuD protein faster than wild-type recA protein [61]. The other biochemically characterized constitutively active proteins, recA1202 and recA1211 proteins, show some similarities to recA441 protein. These proteins have a lower K_m for both ATP and ssDNA; they can use rRNA and tRNA as cofactors for *lexA* cleavage; and they have a relaxed requirement for the NTP needed [62, 63].

In summary, considering the complexity of the reactions, there is good correlation between the *in vivo* and *in vitro* behavior of mutant recA proteins. The quantitative differences that do exist are likely to reflect the difficulty of reproducing the physiologically significant nucleic acid cofactor, ratio of SSB protein to ssDNA, and NTP composition *in vitro* [65]. The specific inducing signal remains somewhat unclear and experimental support exists for either ssDNA [59], dsDNA [66], or RNA [62–64]. Though dsDNA or RNA may play a role in the induction pathway for some of the constitutive proteases, this hypothesis does not explain the additional cleavage that occurs upon DNA damage [67], unless these effectors only partially activate recA protein and full activation requires a second damage-induced effector. For wild-type protein, the most likely inducing signal produced by UV-irradiation is ssDNA gaps resulting from stalled DNA replication forks [59]. This ssDNA is likely to be bound with SSB protein and to be of limited length [42], suggesting that the cellular behavior of recA protein-dependent protein cleavage is represented best *in vitro* by either short ssDNA substrates and/or ssDNA coated with SSB protein. Though unequivocal evidence in support of this conclusion is still not available, further detailed biochemical studies with mutant recA proteins will undoubtedly clarify the details of this important process.

Structure–function relationship

Physical-chemical analysis of the interaction of mutant recA proteins with ATP and DNA provides information on the relationship between recA protein primary sequence and function. To permit discussion and further correlation, the potential functional domains within recA protein are identified and the locations of recA protein mutations are summarized (fig 3).

Nucleotide binding site

The recA protein monomer binds one molecule of NTP or NDP [68, 69], presumably at the same site since the binding is competitive [70]. Direct identification of the amino acid residues comprising the ATP binding site comes from crosslinking experiments. Using 8-azido-ATP and 5'-p-fluorosulfonyl-benzoyladenosine, a peptide containing residues 257–280 was identified, with the 8-azido-ATP crosslinked primarily at tyrosine-264 [71–74]. In contrast, UV-induced crosslinking with ATP identifies a peptide in the region of residues 116–170 [75]. Finally, 8-azido-ADP crosslinks primarily to the peptide containing residues 106–134 and $\approx$ 5-fold less to residues 136–152 [83]. Though the diversity of regions identified may result from differences in the chemistries of the reactive species and/or from differences in conformation of the recA protein-nucleotide complex, they serve to define potential sites of interaction with the sugar and base constituents of the nucleotide. Consistent with the 2 latter studies, chemical modification of cysteine residues 90 and 129 is reduced by ATP binding [77], though the effect may be conformational. RecA protein also contains a consensus sequence for an A-type ATP binding site, G/AXX-XXGKT, starting at residue 66 [78]; crystallographic results show that, in the ras p21 protein, this sequence is involved in interactions with the β–γ phosphate moiety of the nucleotide [79]. Thus, biochemical studies implicate 3 regions of the protein as being part of the ATP binding site: the nucleotide phosphate groups likely bind to the region comprising amino acids 66–73 and the sugar-base component binds to regions 106–170 and 257–280.

DNA binding site(s)

The DNA binding site(s) of recA protein is not well-defined experimentally. Although there are no published crosslinking data, a similarity in the distribution of aromatic and basic amino acid residues between recA protein and other ssDNA binding proteins was noted [80]. This potential ssDNA binding site is located from residues 225 to 312, with aromatic residues phe-255, tyr-264, tyr-271, and tyr-291, basic residues arg-243, lys-248, and lys-310, and acidic residue glu-281 implicated as being involved in ssDNA binding. Based on NMR studies with filamentous phage ssDNA binding proteins, a
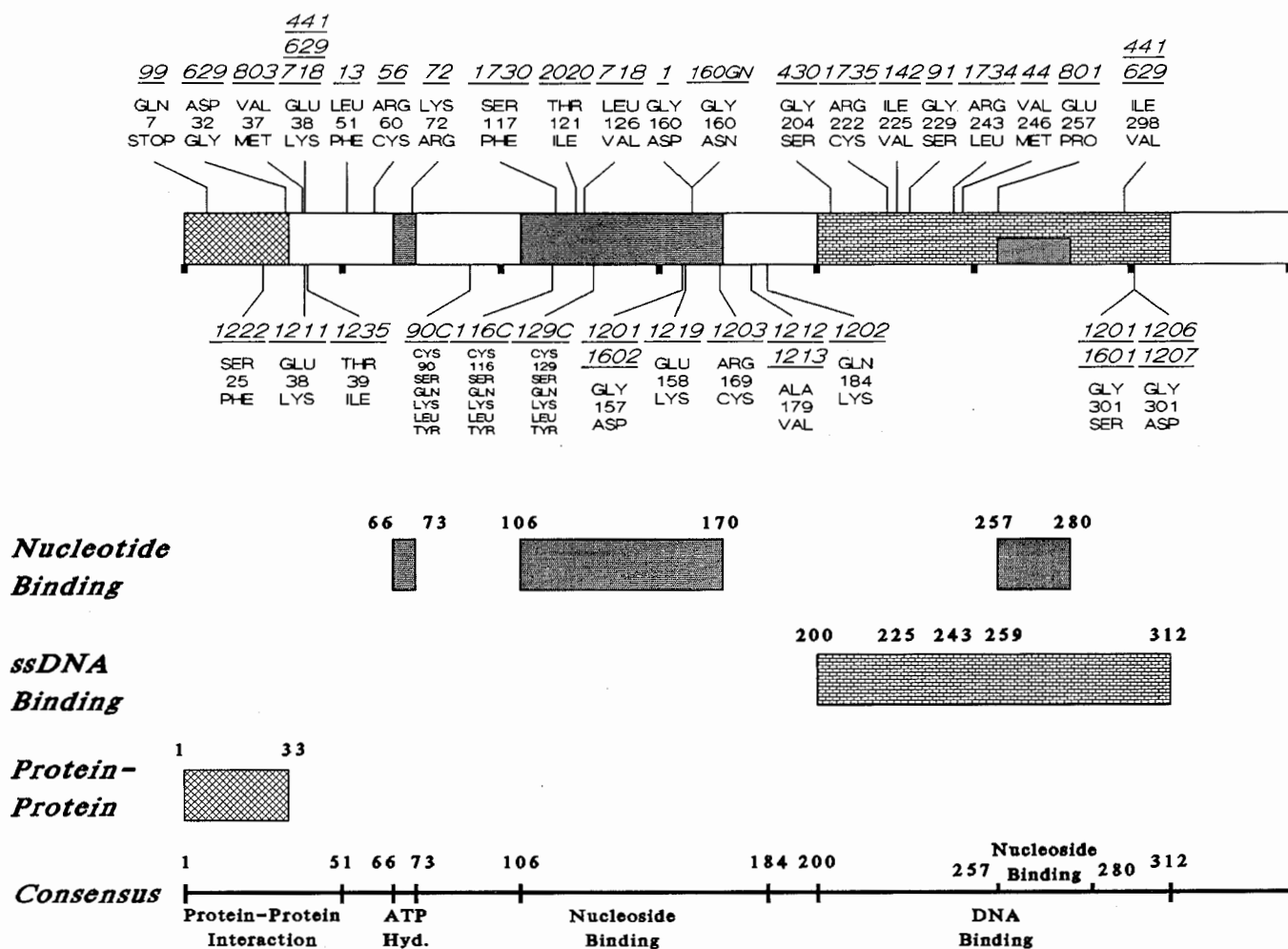
Fig 3. Primary sequence location of recA mutations and of likely functional domains. The references for the sequence data are: *1*, [93]; *13*, AJ Clark, personal communication; *44*, [93]; *56*, AJ Clark, personal communication; *72*, BM Rehrauer and SC Kowalczykowski, unpublished results; *91*, [83]; *99*, [89]; *142*, [89]; *160GN* [50]; *430*, [93]; *441*, [94]; *453*, [89]; *629*, [94]; *718*, [95]; *801*, AJ Clark, personal communication; *803*, [34]; *1201, 1202, 1203, 1206, 1207, 1211, 1212, 1213, 1219, 1222, 1235, 1601, 1602*, [92]; *1730, 1734, 1735*, [89]; *2020*, AJ Clark, personal communication; mutations of the cysteine residues are designated *90C, 116C*, and *129C* [96]. The references for the functional domains are cited in the text.

portion of this region (243–259) is proposed to form a β-loop structure referred to as a 'DNA binding wing' and that is involved in ssDNA binding [81]. The significance, if any, of the overlap of this region with the site identified by 8-azido-ATP crosslinking is nuclear. A similarity between amino acid residues 4–34 of recA protein and the amino terminus of both T4-phage gene 32 protein and SSB protein was also noted [82, 83]; however agreement as to the existence of this similarity is not universal [80]. It was argued that this sequence similarity (if real) defined a ssDNA

binding domain of recA protein; however, this is unlikely because, in gene 32 protein, the N-terminus of the protein is involved in protein–protein interactions important for cooperativity and is not directly involved in ssDNA binding [84]. A similar function for the N-terminus of recA protein is probable.

Some experimental information on DNA binding domains is provided by N-terminal and C-terminal deletion studies. Deletion of 33 amino acid residues from the N-terminus results in a protein that fails to bind ssDNA [83]. Though this may imply that the N-

terminus comprises part of the ssDNA binding domain, failure to bind ssDNA could also result from the loss of cooperative protein–protein interactions [84]. This alternative interpretation is favored because removal of this region results in diminished self-association of recA protein [83]. Removal of the C-terminus (up to ~50 residues), does not attenuate ssDNA binding; in fact, dsDNA binding is somewhat enhanced [26, 85]. Finally, the 150 amino acid C-terminal peptide produced by cyanogen bromide cleavage can bind both ssDNA and dsDNA [86]. Thus, although considerable uncertainty remains, the DNA binding site of recA protein is located approximately between residues 200 to 300. The amino terminus may contain elements of the DNA binding domain, but it is more likely that this region of the protein is involved in protein–protein interactions that stabilize the protein–DNA interactions through cooperativity.

Protein–protein interactions sites

Self-assembly and ssDNA binding cooperativity between monomers requires protein–protein inter-actions. As mentioned above, removal of 33 amino acid residues from the N-terminus reduces protein self-assembly [83]. The participation of the N-termi-nus in self-association is consistent with *in vivo* data obtained with truncated RecA protein fragments. A fragment containing as little as ~77 residues of the amino terminus is sufficient to inhibit recA protein function *in vivo* [87]; although several interpretations are possible, the most likely is that these N-terminal fragments interfere with assembly of the intact recA protein, suggesting that at least one site for protein–protein interactions resides in the N-terminus. The most concrete evidence for the importance of the N-terminus in protein–protein interactions comes from recA protein-affinity column studies [88], which demonstrated the need for a hydrophobic domain within the N-terminal 90 amino acids of recA protein. Thus, these experiments argue that the determinants important for protein–protein interactions reside in approximately the first 33 amino acid residues.

Repressor binding site

Repressor cleavage requires formation of an ATP-recA protein-ssDNA complex, in addition to repressor binding. Since an alteration of any of the former inter-actions will affect repressor cleavage, unambiguous identification of the repressor binding domain by genetic studies is difficult, and quantitative compara-tive *in vitro* analysis is lacking. A differential loss of cleavage activity would be one hallmark of an altered repressor binding site, particularly if cleavage of the most labile substrate, lexA protein, is reduced. A

mutant *recA* allele with such a phenotype is *recA1730* and its mutation is at position 117 [89]. A second candidate mutation is *recA432* [67], but the site of its mutation is unknown. Unfortunately, the studies with all other mutant proteins suffer from at least one potential ambiguity.

Relationship to mutant protein biochemical properties

Interpretation of the binding and kinetic behavior of mutant recA proteins is complicated by the existence of at least 2 different conformations of recA protein (high-affinity and low-affinity states), with transition between the 2 forms being controlled by both the concentration and nature of the effector ligand (*ie* recA protein displays classical allostery; [10]). Thus, a mutant protein with a lower apparent K_m or K_d for ATP can be mutated either in the ATP binding site or at a site involved in the ATP binding-dependent struc-tural transition. Although such uncertainty introduces ambiguity into certain interpretations, some conclu-sions are possible.

Despite the near-total absence of activity for recA1, recA56 and recA13 proteins, the 2 former proteins bind ssDNA nearly as well as wild-type protein, and ADP induces the appropriate low affinity state for recA56 protein [25, 26] (SD Lauder and SC Kowalc-zykowski, unpublished results); hence, both the NTP binding site and the ssDNA binding site of the low-affinity conformation are (relatively) unaltered. In contrast, the affinity of the recA13 protein-ssDNA complexes is greatly reduced, implying that either direct interactions with DNA or cooperative protein–protein interactions are disrupted. For all 3 proteins, however, the affinity of the ATP-bound species is drastically reduced. This could result from either an alteration of the ssDNA binding site of the ATP-induced protein conformation, a flawed struc-tural transition, or a reduction of protein–protein inter-actions responsible for filament stability due to an aberrant conformation. The latter interpretation is plausible for recA13 protein, because it fails to form mixed filaments with wild-type recA protein (SD Lau-der and SC Kowalczykowski, unpublished results) and because the mutation is close (residue 51) to the crucial N-terminal protein–protein interaction domain. For recA1 and recA56 proteins, the alternative inter-pretation of an impeded structural transition that is coupled to ATP binding is a reasonable possibility, given the proximity of the mutations (residues 60 and 160) to ATP binding domains and the fact that both proteins interact with wild-type recA protein ([90], SD Lauder and SC Kowalczykowski, unpublished results).

RecA142 protein has an 8-fold higher K_m for ATP than wild-type protein and fails to completely induce the characteristics of the high affinity state [27]. In the

absence of nucleotide cofactor or in the presence of ADP, its ssDNA binding properties are identical to wild-type protein. This implies that both the nucleotide binding site itself and the ssDNA binding site of the low affinity conformation are unaltered. However, in the presence of ATP, the STMP and the RFI are lower than for wild-type. Together these results imply that the higher apparent K_m and the lower ssDNA affinity results from a defective structural transition. Consistent with this interpretation, the potent effector ATPγS induces the high-affinity state of recA142 protein. Thus, amino acid residue 225 is probably involved in the ATP-dependent structural transition to the high-affinity state, despite being located in the loosely-defined DNA binding domain.

The recA430 protein differs from the DNA strand exchange-defective proteins in one significant respect: both ATP and dATP are capable of inducing the high affinity state [30]. Though the stability of the ATP–recA430 protein–ssDNA complex is lower than that of wild-type protein, the RFI values are equal. In addition, the K_m for ATP is only 1.4-fold greater, suggesting that there is relatively little alteration in either the ATP binding site or the ATP-dependent conformation change. Also like the recA142 protein, the ssDNA binding properties in the absence of nucleotide and presence of ADP are quantitatively unaltered, demonstrating that the nucleotide binding site and the low affinity ssDNA binding site are unaltered. Thus, residue 204 is in a region responsible for the stability of the high affinity conformation with ssDNA; this can be part of either the ssDNA binding site of the high affinity conformation or the protein–protein interaction site.

The *recA441, recA803*, and *recA1211* mutations are adjacent to one another (residues 38 and 37; fig 3); in addition, *recA441* has a second mutation at residue 298. Genetic data suggest that the latter mutation confers the temperature-dependent of *recA441* behavior [91]. Consequently, the similarity in the physical properties of these 4 mutant proteins is most likely due to the mutations at residues 37 and 38. Since the ssDNA binding properties of these proteins are almost identical to that of wild-type protein, it is unlikely that either the NTP or the ssDNA binding sites are located near these residues. However, both recA441 and recA803 proteins associate with ssDNA more rapidly than wild-type protein, suggesting that residues 37 and 38 comprise a site for protein–protein interactions that are crucial for the rate-limiting nucleation event. The importance of this domain of recA protein was previously highlighted by the clustering of protease-constitutive mutations at residues 25–39, 157–184, and 298–301 [92]. In agreement with biochemical data, the first region was proposed to be involved in protein–protein interactions and the second in nucleo-

tide binding; the function of the third region remains obscure.

To directly examine the role of the predicted ATP consensus sequence, the recA72 protein was engineered by site-specific mutation of residue 72. This amino acid is an invariant residue in the ATP consensus sequence [78] and it was changed from lysine to a conservative arginine (BM Rehrauer and SC Kowalczykowski, unpublished results). ATP and dATP hydrolysis by this protein is reduced by > 600-fold, yet still retains NTP binding, DNA strand exchange, and lexA repressor cleavage activities. The ssDNA binding properties of recA72 protein in the absence of nucleotide or in the presence of dATP are virtually identical to those of wild-type protein. Thus, the properties of this mutant protein verify the identification of this domain as part of the catalytic center for NTP hydrolysis.

Taken together, the chemical crosslinking, protein sequence comparison, and biochemical genetic results suggest the consensus assignments shown in figure 3. The protein–protein interaction domain is located at the N-terminus and extends for 40–50 amino acid residues. The nucleotide binding domain consists of 3 separate regions: residues 66–73 comprise the catalytic domain, and residues 106 to possibly 184 and residues 257 to 280 comprise the nucleoside binding domains. The DNA binding domain is difficult to assign, but is probably located between residues 200 and 312. Unfortunately, the repressor binding domain is even more uncertain, being defined (unambiguously) by a mutation at amino acid residue 117.

Conclusions

Biochemical characterization of mutant recA proteins demonstrates a clear correlation between *in vivo* phenotype and *in vitro* properties. All of the mutant recA proteins that are devoid of genetic recombination *in vivo* are also deficient in both DNA strand exchange and DNA renaturation activities; all of the mutant proteins that show reduced recombination activity also show reduced DNA strand exchange and DNA renaturation activities; finally, all of the mutant recA protein that are 'enhanced' for an *in vivo* recombination function also have enhanced homologous pairing activities *in vitro*.

The one biochemical property of recA protein that predicts both the biological and the biochemical behavior of a mutant recA proteins is the ability to compete with SSB protein for ssDNA binding. All mutant recA proteins that can displace SSB protein are also proficient in DNA strand exchange, and all mutant proteins that are displaced by SSB protein fail to promote DNA strand exchange (even in the absence of SSB protein). The competition with SSB

protein is also important to the repressor cleavage reactions because, where examined, the phenotype of some mutant recA proteins is more consistent with *in vitro* repressor cleavage activity in the presence of SSB protein than the activity in the absence of SSB protein.

The ability of recA protein to compete with SSB protein is related to its ssDNA binding affinity relative to the ssDNA binding affinity of SSB protein. Consequently, a change in SSB protein displacement ability reflects an underlying alteration in the interaction of recA protein with ssDNA. This interaction is characterized by 2 alternate DNA binding states. Induction of the high affinity state is absent in all of the mutant recA proteins displaying a null phenotype and is impaired in those displaying limited *in vivo* and *in vitro* function. Thus, functionality in both genetic recombination and DNA strand exchange activity require formation of the high affinity binding state of recA protein. The high affinity of recA protein is essential in the transduction of the free energy of ATP binding to the free energy required for processes such as SSB protein displacement and the opening of dsDNA during DNA strand exchange [10]. The enhanced SSB displacement activity of certain mutant recA protein is explained only by the increased rate of association with ssDNA, arguing that the rates of the relevant kinetic steps of the displacement process are critical elements of this dynamic system. Further characterization of mutant recA proteins is sure to resolve many of the remaining biological and biochemical issues.

Acknowledgments

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