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THE recA PROTEIN OF *E. coli*: REGULATION AND FUNCTION IN RECOMBINATION AND REPAIR

(UV induction, autoregulatory model, DNA binding protein, DNA renaturation, strand assimilation)

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SUMMARY

In Escherichia coli, the recA function is required for general recombination, repair of DNA damage and a diverse group of functions which are coordinately expressed following DNA damage or arrest of DNA synthesis (SOS functions). These latter functions include UV mutagenesis, W-reactivation of damaged phage DNA, prophage induction and cell filamentation.

In this paper we summarize experiments which have elucidated regulatory and functional aspects of the recA gene product. Biochemical and genetic data suggest a model for control of recA gene expression in which the recA protein autoregulates its own synthesis following UV irradiation or other forms of DNA damage. The features of this autoregulatory mechanism are different from those proposed for gene 32 protein of phage T4. Biochemical experiments with homogeneous wild type and mutant forms of RecA protein reveal that this unique DNA binding protein catalyzes DNA renaturation and single strand assimilation reactions which are coupled to ATP hydrolysis. These results indicate that RecA protein catalyzes strand transfer during the initiation of genetic recombination and during post-replication repair of damaged DNA in vivo.

INTRODUCTION

The *rec*A gene of *Escherichia coli* performs a central role in genetic recombination, DNA repair, and in several other important aspects of normal DNA metabolism. Mutations in this gene produce a complex pleiotropy which includes extreme recombination deficiency (typically 10^{-2}-10^{-4}% of wild type levels), decreased survival following UV irradiation or other DNA damaging treatments, and complete loss of UV-mutability as well as temperate prophage inducibility. A loss of *rec*A function also results in altered cell division properties and excessive DNA degradation following DNA damage (see CLARK 1973 and WITKIN 1976 for reviews).

The original *rec*A mutants were defective in all of these processes. Recently, several additional mutations have been located in or very close to this gene (at minute 58 of the *E. coli* linkage map) which are partially defective or hyper-expressive for functions controlled by *rec*A. In addition, deletion and insertion mutations in the *rec*A gene have been obtained. A partial list of these alleles and their phenotypes is given in Table I. Several of these mutations have been shown to map in the *rec*A gene by two and three factor P1 *kc* transductional crosses as well as by complementation tests with F' elements (MORAND et al. 1977b). Physical evidence has been obtained that the *tif*-1, *rec*A12 and *rec*A629 mutations lie within the *rec*A structural gene (McENTEE 1977). In light of the genetic and physiologic complexities of these mutations it is rather surprising that the protein product of this gene is a relatively small polypeptide with a molecular weight of 40,000 (McENTEE et al. 1976).

Table I

Phenotypic Properties of recA Alleles

recA Allele	Phenotype	Remarks
recA1 recA12 recA13 recA56 recA99 recA123	Full RecA$^-$ phenotype: recombination deficient (Rec$^-$), UV^S, no reactivation, prophage induction or UV mutagenesis.	Missense mutation altered isoelectric pt. Small deletion in *rec*A gene. Amber mutation. Possible nonsense mutation.
recA142	Rec$^-$, UV^S	Normal levels of spontaneous prophage induction
lexB30	Rec$^+$, UV$^{R/S}$	No prophage induction; *tsl* suppresses UV sensitivity and RecA protein induction.
zab53	Rec$^+$, UV$^{R/S}$	Isolated as suppressor of *tif*-1; no RecA protein made in *tsl zab*53 mutant; isoelectric pt. same as *tif*-1.
recAΔ21 recAΔ7	Full RecA$^-$ phenotype	Deletions extending from *srl*A gene into recA gene.
recA629	RecA$^-$ at 30°C RecA$^+$ at 42°C	Cold sensitive RecA$^-$ phenotype; derived from *tif*-1; isoelectric pt. variant.
tif-1	Rec$^+$ but hyperinducible for SOS functions	Isoelectric pt. variant of RecA protein.
recA(Mu)	RecA$^-$ phenotype	Insertion of phage Mu into *rec*A gene (P. KUSHNER, personal communication).

Insight into the function of the *rec*A gene in DNA metabolism has come from investigations of the regulation of this gene *in vivo* and from the enzymatic activities of the RecA protein *in vitro*. In this paper we summarize results of some of these experiments and their implications for the role of RecA protein in the processes of general recombination and postreplication repair in *E. coli* .

RecA PROTEIN IDENTIFICATION

Failure to find an enzymatic defect in extracts prepared from *rec*A mutant cells frustrated early efforts to identify the product of this gene (CLARK et al. 1966). Identification of the RecA protein was achieved using a derivative of bacteriophage λ which had incorporated the *rec* A genetic region into its chromosome. The *rec*A gene on the specialized transducing phages was expressed from its own promoter during growth as a prophage or during lytic development. Comparing the proteins made after infection of Su^+ and Su^- hosts by a phage containing an amber *rec* A mutation indicated that suppression of the nonsense codon resulted in the appearance of a single protein with a molecular weight of approximately 40,000 (McENTEE et al. 1976). The mobility of the RecA protein on polyacrylamide gels containing sodium dodecyl sulfate was found to be identical to that of a protein, called protein X, which is induced in cells following UV irradiation or other treatments which damage DNA or arrest DNA synthesis (INOUYE and PARDEE 1970). The identity of this UV inducible protein as the product of the *rec*A gene came from several observations;

1) Mutations in the *rec*A gene such as *rec*A1 or *tif*-1 altered the isoelectric properties of the protein X of the mutant strain (GUDAS and MOUNT 1977; McENTEE 1977).

2) An electrophoretic variant of protein X was shown to be produced in strains carrying the *rec*A12 mutant allele (McENTEE 1977).

3) Expression of the $rec A^+$ gene of the transducing phage in certain mutant hosts was analogous to expression patterns of the X proteins in these mutants (McENTEE 1978; SEDGWICK et al. 1978).

4) The one dimensional tryptic and chymotryptic digestion patterns of the RecA protein made after infections by the transducing phage were identical to the patterns of fragments obtained after digestion of the partially purified protein X (LITTLE and KLEID 1977).

In Vivo Regulation of the *Rec*A Gene

The implications of this identification are both important and interesting. First, genetic analysis of protein X (RecA protein) induction indicated that at least two genes are required for expression of the *rec* A gene: the

unlinked *lex*A gene as well as the *rec*A gene itself. Both *lex*A$^-$ and *rec*A$^-$ mutants are blocked in RecA protein (protein X) derepression after treatments such as UV irradiation or nalidixic acid inhibition of DNA replication (GUDAS and PARDEE 1975). Second, considerable evidence indicates that the *rec*A controlled processes of UV mutagenesis, W-reactivation (enhanced repair capacity of UV damaged phage DNA), prophage derepression, cell filamentation and other cellular responses to UV irradiation are induced in damaged cells (SOS response). The induction of these functions, like the induction of the RecA protein, requires both *lex*A$^+$ and *rec*A$^+$ genes and protein synthesis (WITKIN 1976). Furthermore, the induction kinetics of at least two of these processes--UV mutagenesis and W-reactivation--are similar to the induction kinetics observed for the RecA protein (DEFAIS et al. 1976; GUDAS 1976). The suggestions from these observations are that the RecA protein participates directly in at least some of the inducible processes and that high levels of the protein are required for these reactions to proceed efficiently. Although the induction ratio for RecA protein has not been accurately determined, the level of the RecA protein is increased approximately 50-500 fold after UV-irradiation and constitutes 2-5% of the soluble cellular protein after such treatment. This copious production of RecA protein is difficult to reconcile with a purely regulatory role for this protein, which had been suggested to account for the pleiotropic effects of mutations in this gene. Indeed, Roberts and co-workers have provided evidence that the RecA protein proteolytically cleaves phage λ repressor (ROBERTS et al. 1978). The protein is required at high levels *in vitro* for repressor cleavage and these authors have suggested that not only is this the likely involvement of RecA protein in prophage induction, but also that RecA protein may activate or modify other proteins which participate in recombination and repair reactions. Although no evidence supports this latter speculation, it is evident, nevertheless, that the extent of *de novo* RecA protein synthesis following UV treatment is considerable and that this induction is important for cell survival.

Two other mutations, *tsl* and *spr*, have been shown to affect RecA protein synthesis. Both mutations result in partial constitutivity for RecA protein synthesis. The former mutation shows a temperature inducible synthesis of RecA protein (GUDAS 1976) and the latter mutation increases the level of RecA protein during growth at all temperatures (McENTEE 1978). Both mutations are located extremely close to or within the *lex*A gene (*tsl* and *spr* were isolated as UV-resistant survivors of a *lex*A$^-$ strain). Taken together, the genetic evidence provides the basis of an autoregulatory model for *rec*A gene expression (FIGURE 1) (McENTEE 1977; GUDAS and MOUNT 1977). The *rec*A gene is repressed during normal growth such that a low basal level of the protein is synthesized in cells. This level is necessary and presumably sufficient for certain constitutive functions including general recombination. Following irradiation, an effector molecule is produced in cells as a consequence of DNA damage. The nature of this effector molecule is unknown at the

present time although it has been suggested that this hypothetical effector is a DNA degradation product, probably a small oligonucleotide, produced by the recBC nuclease (GUDAS and PARDEE 1975). The effector molecule is presumed to bind to the RecA protein and induce a conformational change which converts it into a form (RecA') capable of inactivating the *lex*A coded repressor. Removal of this repressor from the *rec* A operator results in increased transcription of this gene and extensive synthesis of the *rec* A gene product. This scheme, as originally formulated, accounts for many of the genetic observations concerning induction of *rec* A. The *lex*A$^-$ mutants, which are dominant to the wild type *lex* A$^+$ allele, represent a class of non-inducible or super-repressor mutations. The mutant protein may bind more tightly to the operator region (analogous to the i^s mutations in the *lac* I gene) or the protein may have a reduced affinity for the activated RecA' protein. RecA$^-$ mutations produce an altered gene product which cannot be induced by UV irradiation because the abnormal protein fails to bind effector and cannot be activated. Mutations such as *tsl* or *spr* are examples of temperature sensitive and partially nonfunctional repressor mutations, respectively. Induction of RecA protein synthesis by *tsl* or *spr* mutations, unlike UV induction, requires no functional participation of RecA protein. Overproduction of several missense RecA proteins has been achieved in *rec*A$^-$ strains with *tsl* or *spr* mutations. These double mutant strains have been very useful for the purification of RecA protein from several *rec*A$^-$ strains (WEINSTOCK et al. 1979).

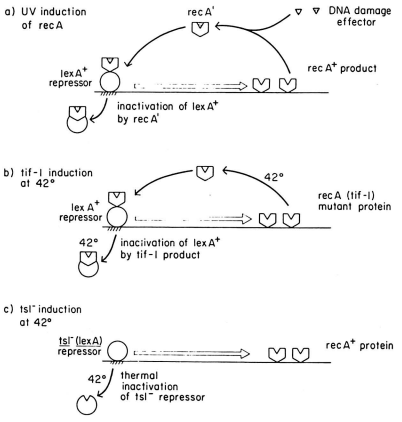

Figure 1. A model for the transcriptional regulation of the *rec*A gene by the *lex*A product and the RecA protein. From McEntee (1977).

The unusual *tif*-1 mutation, alluded to earlier,

expresses the inducible (SOS) functions at 42° without DNA damage (CASTELLAZZI et al. 1972a). The level of RecA (*tif*) protein increases dramatically after temperature shift and this derepression by *tif*-1 expression is blocked by *lex*A$^-$ or *rec*A$^-$ mutations which block UV induction of RecA protein. The *tif*-1 allele is postulated to be a special class of *rec*A mutation, the product of which mimics the properties of the activated RecA' form at high temperature without an effector molecule. It is possible that the RecA (*tif*-1) protein undergoes a conformational change at 42°C, and, in a manner analogous to the activated RecA' protein, removes the *lex*A coded repressor leading to overproduction of the altered RecA protein.

Two additional mutations, *lex*B30 (MORAND et al. 1977a) and *zab*53 (CASTELLAZZI et al. 1972b), result in defective expression of repair, mutagenesis, prophage derepression and other inducible functions but reduce recombination proficiency only slightly. Furthermore, little or no RecA protein is induced in these strains following UV irradiation or nalidixic acid treatment. Although the double mutant *tsl* (or *spr*) *lex*B30 strain overproduces the mutant RecA product, no such overproduction is observed for the *tsl zab*53 strain. A possible explanation for this result is that the *zab*53 mutation is in the control region for the *rec*A gene, perhaps a 'down-promoter' class of defect which would prevent RecA protein induction by any of the pathways described. The *zab*53 mutant protein has been examined by isoelectric focusing and found to have an isoelectric point identical to the parental mutant product. Although additional experiments are needed to identify the nature of the *zab*53 lesion, this mutation should prove extremely useful in analyzing the regulation of the *rec*A gene *in vivo* and *in vitro*.

Features of this autoregulatory model are consistent with several observations. McPartland et al. (McPARTLAND et al. 1978) have measured *rec*A gene specific transcripts by filter hybridization. The rate of transcription of the *rec*A region increases 10-15 fold following UV treatment of *rec*A$^-$ *lex*A$^+$ cells. Strains containing either *rec*A$^-$ or *lex*A$^-$ mutations do not increase transcription of this region following UV irradiation. Thus regulation of the *rec*A gene by its own product and by the *lex*A gene product appears to be at the transcriptional level, consistent with the model.

Multicopy hybrid plasmids which contain the *rec*A$^+$ gene can suppress the UV-sensitivity of *rec*A$^-$ as well as *lex*A$^-$ strains when the plasmids are transferred into these genetic backgrounds (McENTEE 1978). These results suggest that overproduction of RecA protein overcomes the defect in *lex*A$^-$ cells. Based on this model an explanation for these results is that the *lex*A$^-$ repressor is titrated out by multiple copies of the *rec*A operator region. Removal of the repressor would permit elevated expression and synthesis of RecA protein. Additional experiments are required in order to precisely determine the effects of these plasmids on expression of the *rec*A gene *in vivo*. A partial suppression

of the UV sensitivity of *recA*$^-$ mutants has been observed upon introduction of a second *tsl*$^-$ or *spr*$^-$ mutation (MOUNT et al. 1976). The double mutant strains *tsl*$^-$ *recA*$^-$ or *spr*$^-$ *recA*$^-$ are more resistant to killing by UV irradiation than the *tsl*$^+$ (*spr*$^+$) *recA*$^-$ strains (MOUNT et al. 1976). Presumably elevated levels of a 'defective' *rec* A gene product can assist in some unknown way in enhancing cell survival.

UNANSWERED QUESTIONS

The regulatory model for RecA protein induction accounts for many of the *rec*A and *lex*A mutations, as well as the properties of mutants carrying combinations of these lesions. However, several important questions remain concerning the features of such a regulatory scheme. For example, what is the nature of the 'inducer' or effector of the *rec*A gene? Is the effector which causes derepression of the *rec*A gene also responsible for induction of other genes or operons which might be expressed following DNA damage? It has been suggested that the effector is a small molecule, derived from 'idling polymerases' (VILLANI et al. 1978) or from recBC nuclease digestion of DNA following damage (GUDAS and PARDEE 1975; SMITH and OISHI 1978). However, the variety of treatments capable of derepressing the *rec*A gene (nalidixic acid, bleomycin, mitomycin C, *dna*Bts at nonpermissive temperature) do not produce the same types of lesions which might cause a polymerase to idle as is observed at a pyrimidine dimer. The fact that RecA protein is induced in *recBC*$^-$ mutants by UV or bleomycin treatments indicates that digestion by the recBC nuclease cannot be the only mechanism by which an effector molecule is generated. Perhaps a more likely inducer is single-stranded DNA, which may be generated transiently after most of these diverse treatments. The RecA protein binds to single-stranded DNA and this interaction may reflect a regulatory role for the single-stranded DNA in addition to serving as a substrate in repair/recombination reactions. A DNA binding protein of bacteriophage T4, the product of gene 32 (P32), is inducible by blocking DNA replication (KRISCH et al, 1974). Synthesis of this protein is regulated *in vivo* by the amount of single-stranded DNA produced in the cell as well as by P32 itself. A model for P32 regulation has been proposed in which the single-stranded DNA and a regulatory site compete for the binding protein. Titration by excess single strands reduces the level of free P32 available for repression of P32 synthesis and causes increased synthesis of the gene 32 product (GOLD et al. 1976). Moreover, the single stranded DNA is a presumed substrate for recombination and repair functions of P32. At the present time there is no direct evidence implicating single-stranded DNA in the regulation of RecA protein synthesis. Experiments are in progress to test this possibility.

The regulatory model as presented originally (McENTEE 1977; GUDAS and MOUNT 1977) does not take into account the possible involvement of other genes in the induction of RecA protein. Recent evidence suggests that at least two

additional loci, *rec*F (HORII and CLARK 1973) and *lex*C (JOHNSON 1977) affect the kinetics or extent of RecA protein synthesis. Whether these genes participate directly or indirectly in induction is unknown. The possibility that these genes may be involved in post-transcriptional regulation of *rec*A cannot be ignored.

As depicted in Figure I, the positive feedback induction loop is unstable--there is no mechanism for the 'shut off' of the *rec*A gene once the repressor has been removed or inactivated after induction. One way of reestablishing a repressed state is inactivation of the RecA protein by degradation or modification. Measurements of the chemical stability of RecA protein indicate that the protein is stable in the cell and is lost by dilution during cell division. This long chemical half-life for RecA protein is to be contrasted with the much shorter half-lives characteristic of the inducible SOS functions of mutagenesis and W-reactivation which decay within a period of approximately 30 minutes (DEFAIS et al. 1976). One explanation is that the RecA' intermediate is unstable or is consumed in the repressor inactivation step. If RecA' is unstable, this form of RecA protein may be responsible for mutagenesis, prophage induction and other SOS functions.

Another regulatory feature yet to be demonstrated is whether transcription of the *rec*A gene is initiated at two promoters: a 'weak' promoter for constitutive expression of the gene and a 'strong' promoter, controlled by the *lex*A product, which could be used for initiating transcription following induction.

Answers to these questions await a detailed analysis of *rec*A gene expression *in vitro* as well as sequence information for the important regulatory regions of the *rec*A gene.

BIOCHEMISTRY OF THE RecA PROTEIN

Biochemical and enzymatic studies of the RecA protein are facilitated by the overproduction of this protein in cells that carry an *spr* mutation or have been treated with nalidixic acid. Using a simple three step purification procedure, we have purified RecA proteins from *rec*A$^+$, *lex*B30, *tif*-1, and *rec*A629 mutant strains (the *rec*A629 allele renders cells cold sensitive for recombination and UV survival--at 30°C the strain is phenotypically RecA$^-$ although at 42°C the strain is recombination proficient and resistant to killing by UV irradiation). The nearly homogeneous proteins (greater than 95% pure) catalyze hydrolysis of ATP, dATP, UTP and dUTP, consistent with a preliminary report by Ogawa (OGAWA et al. 1978). The ATPase and dUTPase activities copurify with the RecA proteins. In the case of the RecA protein purified from the cold-sensitive recA629 strain, the ATPase has been shown to be cold labile (WEINSTOCK et al. 1979). The recA protein purified from *rec*A mutant strains displaying markedly different SOS responses (*lex*B30 and *tif*-1) also hydrolyze ATP, although somewhat less efficiently than the wild-type

protein.

The ATPase of RecA protein requires single-stranded DNA as a cofactor. In the absence of DNA no hydrolysis of ATP or dUTP can be detected (<2%). At very high concentrations of RecA protein, ATP is hydrolyzed by the enzyme in the presence of duplex DNA.

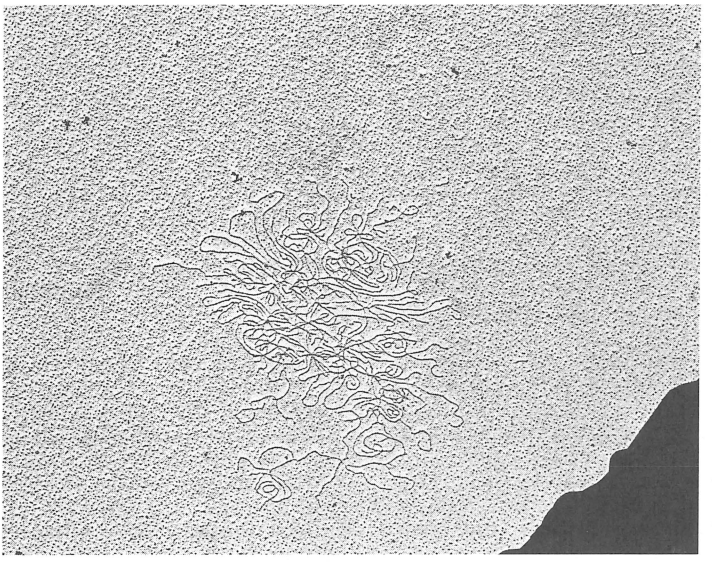

Figure 2. A renatured colE1 DNA complex formed in the presence of RecA protein and ATP. From Weinstock et al. (1979).

Incubation of RecA protein, heat denatured DNA and ATP results in the formation of large DNA aggregates which contain both single stranded and duplex regions as demonstrated by electron microscopic examination of the product (Figure 2) and digestion with the single strand specific nuclease S1. More than 50% of the DNA becomes resistant to S1 nuclease digestion indicating extensive renaturation of the DNA. The complexes of DNA are multiply branched structures, indicating that local annealing of a single strand to several complementary strands at different sites is occuring in the reaction. These products are formed with large DNA (P22, λ) as well as with relatively small DNA (colE1, ϕX174) that has been denatured. In the absence of RecA protein, there is no formation of these complexes and only a small amount (typically less than 5%) of uncatalyzed renaturation. Although we can detect a slow ATP independent renaturation of DNA in the presence of RecA protein, the rate of annealing is stimulated markedly (between 5-10 fold) by ATP. Inhibitor studies and competition experiments indicate that renaturation of DNA catalyzed by RecA protein is tightly coupled to ATP hydrolysis.

The RecA protein catalyzed annealing of free single strands differs from the renaturation of DNA promoted by P32 of phage T4 or the *E. coli* binding protein (or helix destablizing protein) in at least two fundamental ways. Neither P32 nor *E. coli* binding protein efficiently promotes renaturation when DNA is in large molar excess (in terms of nucleotides) compared to protein. Saturation of the DNA with P32 or binding protein accelerates renaturation presumably because secondary structure of the DNA is removed by protein binding. We have observed efficient renaturation catalyzed by RecA protein when DNA is in large excess: 500 nucleotides per monomer of protein. Neither P32 nor *E. coli* binding protein promoted renaturation requires ATP hydrolysis as does the RecA protein catalyzed reaction. Inhibition of recA protein catalyzed hydrolysis of ATP by analogs or NEM, blocks renaturation (WEINSTOCK et al. 1979). We conclude that the mechanism for DNA renaturation by RecA protein is fundamentally different from other binding proteins. Furthermore, the RecA629 mutant protein is cold-labile for catalysis of single-stranded DNA renaturation--a result which strongly implicates this reaction in the processes of recombination and repair *in vivo*.

A variety of genetic and biophysical results suggest that an important intermediate in genetic recombination is a heteroduplex region of DNA in which one strand of the duplex is contributed by each parent. This 'heteroduplex joint' is formed at an early step in recombination in *rec*A$^+$ cells. In *rec*A$^-$ mutants, heteroduplex structures are not detected as early recombinational intermediates. The demonstration that RecA protein catalyzes formation of duplex DNA from single strands *in vitro* provides evidence that the role of this protein in recombination is catalyzing formation of the heteroduplex region from the parental DNA molecules. Such a role in the formation of heteroduplex molecules explains the absolute requirement for *rec*A function in all pathways of general recombination.

The annealing activity of RecA protein suggests a role for this protein in repair of DNA as well. Following UV irradiation, DNA synthesis is blocked at unexcised pyrimidine dimers. Large single stranded regions (gaps of 1000 nucleotides or more) accumulate in the partially replicated daughter strand which are not filled in by polymerases due to the persistence of the dimer. Post-replication filling of these gaps has an absolute requirement for *rec*A function *in vivo* (SMITH and MEUN 1970). Annealing of an intact complementary strand into these damaged regions catalyzed by RecA protein provides a mechanism for generating an intact strand for replication and segregation of an undamaged chromosome. It is likely that induced levels of RecA protein which accumulate following UV treatment accelerate the gap filling mechanism and permit DNA replication to resume.

Although the reactions described above for RecA protein involve single-stranded DNA, we have recently observed that RecA protein interacts with duplex DNA. The results of these investigations are summarized below.

1) RecA protein binds cooperatively to duplex DNA in the presence of a purine nucleoside triphosphate. The binding reaction does not require hydrolysis of the nucleoside triphosphate.

2) A stable RecA protein-DNA complex can be detected in the presence of GTP. In the presence of ATP, complexes are formed and upon further incubation the RecA protein dissociates from the duplex. ATP, unlike GTP, is hydrolyzed in the reaction with duplex DNA and RecA protein.

3) Incubation of RecA protein, duplex DNA, ATP (but not GTP) and homologous single strands results in assimilation of the single-stranded DNA into the duplex. Assimilation produces displacement loop (D-loop) structures which are formed efficiently in linear and superhelical DNA.

4) The RecA629 mutant protein is cold labile for the assimilation reaction.

These results indicate that RecA protein catalyzes a single strand assimilation reaction which is implicated in the initiation of general recombination (see RADDING 1978). The relationship of this strand assimilation reaction and the annealing of free single strands is depicted in Figure 3.

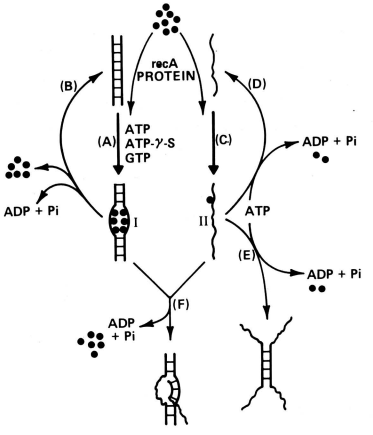

Figure 3. A scheme depicting the renaturation and assimilation reactions catalyzed by the RecA protein. See text for details.

Binding of RecA protein to duplex DNA is cooperative and requires a purine nucleoside triphosphate (ATP, GTP, ATP-γ-S promote binding) but does not require hydrolysis of the triphosphate (reaction A). The RecA protein-DNA intermediate which is formed probably contains partially denatured

regions. RecA protein may unwind the helix directly upon binding to the duplex, or alternatively, the protein might bind to single stranded regions created during breathing of the duplex, destabilize adjacent duplex regions and promote additional binding of the protein, accounting for the cooperativity. If ATP is the purine triphosphate cofactor for binding, it can be hydrolyzed by the RecA protein-DNA complex (single-stranded DNA being present) leading to the release of the RecA protein from the DNA and rapid renaturation of the duplex (reaction B).

RecA protein binds to single-stranded DNA in the absence of purine nucleosides triphosphates and in a noncooperative fashion (reaction C). In the absence (reaction D) or presence (reaction E) of a complementary single strand, RecA protein hydrolyzes ATP. The latter reaction leads to the annealing of the complementary strands as described earlier. The annealing of a single strand into homologous duplex DNA occurs when a RecA protein-DNA complex is incubated with ATP and single strands (reaction F). The single strands are hybridized to the denatured portion of the duplex resulting in hydrolysis of ATP and release of RecA protein.

The requirement for ATP hydrolysis during renaturation of DNA and the requirement for a purine nucleoside triphosphate in the binding of RecA protein to duplex DNA have been examined in detail. Our evidence indicates that the binding of a purine nucleoside triphosphate to RecA protein induces a conformational change which increases the affinity of the protein for DNA. If the triphosphate can be hydrolyzed by RecA protein (as in the case of ATP) then the hydrolysis of the ATP to ADP reduces the binding affinity of the protein and promotes dissociation of RecA protein from DNA. When GTP or ATP-γ-S is used as cofactor in the binding reaction, no hydrolysis is observed and a stable RecA protein-DNA complex is formed. The properties of this complex are presently being investigated.

CONCLUDING REMARKS

The RecA protein is a unique DNA binding protein. Similar to P32 of phage T4, RecA protein synthesis is autoregulated *in vivo* and is induced by treatments which damage DNA although certain features of the regulatory mechanisms differ for the two proteins--RecA protein synthesis is regulated at the transcriptional level, P32 is post-transcriptionally regulated. The RecA protein, like P32 and the *E. coli* binding protein, promotes DNA renaturation. Unlike the other single-stranded DNA binding proteins, ATP is required in the renaturation reaction catalyzed by RecA protein. Hydrolysis of ATP releases RecA protein from DNA during renaturation and permits cycling of the protein. This allows RecA protein to catalyze renaturation when DNA is in tremendous excess. The requirement for a purine nucleoside triphosphate in the binding of RecA protein to duplex DNA provides evidence for allosteric effects upon this protein.

Preliminary experiments indicate that the single-stranded DNA binding properties of RecA protein are markedly affected by both purine and pyrimidine nucleoside triphosphates. We are presently attempting to relate the effects of certain mutations in the *rec*A gene to these binding properties in order to understand the function of this protein in UV mutagenesis and other *rec*A dependent processes.

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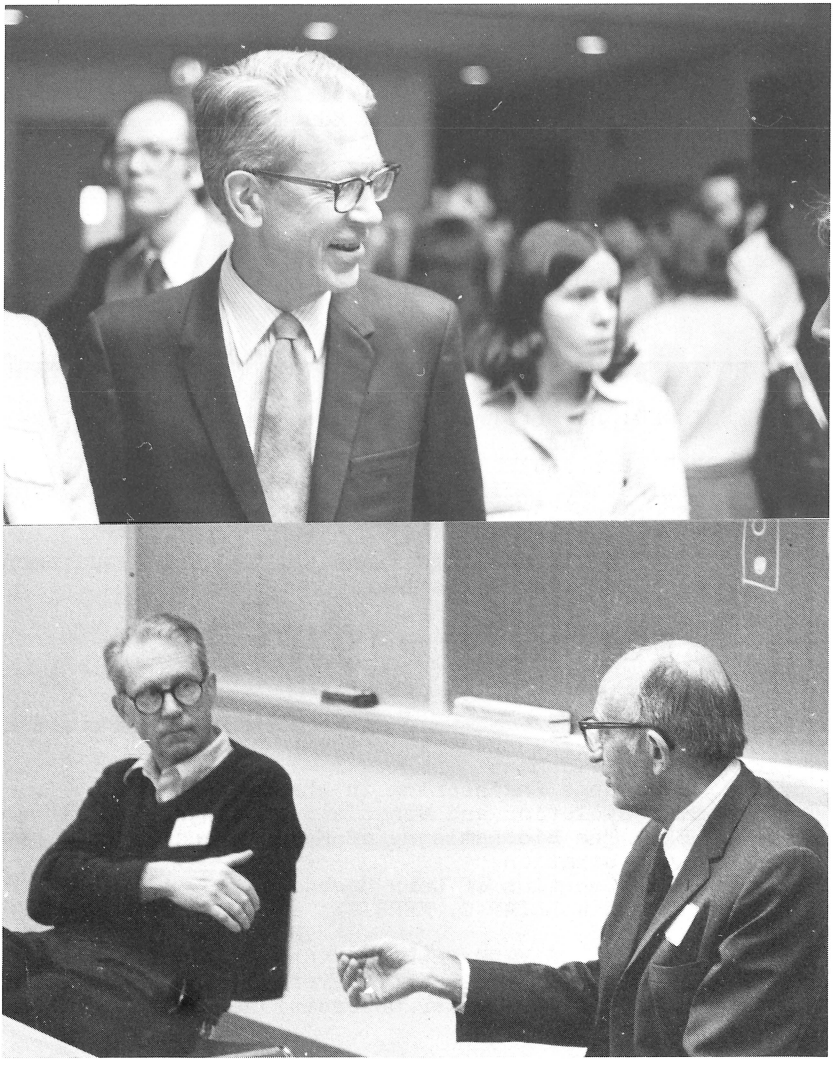

Dr. Perkins (top and lower left) and Dr. Sears (lower right).