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RecA protein

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Introduction

The RecA protein of *Escherichia coli* is a multifunctional protein that is essential to three distinct, but related biological processes: (i) general genetic recombination; (ii), regulation of the co-ordinated expression of many unlinked genes in response to DNA damage, known as the SOS response; and (iii) the error-prone replicative bypass of DNA lesions, resulting in a highly mutagenic repair of DNA. Not surprisingly, mutations in *recA* are pleiotropic, affecting not only recombination, but also DNA repair, mutagenesis, and cell division.

To mediate this broad spectrum of biological events, RecA protein possesses three major biochemical activities: (1) the homologous pairing and exchange of DNA; (2) ATP and DNA-dependent co-proteolytic processing of effector proteins; and (3) interaction with mutagenic protein factors to facilitate error-prone DNA synthesis past DNA lesions.

The DNA strand exchange activity is characteristic of a class of proteins that are essential to genetic recombination, a biological process in which two homologous DNA molecules pair and exchange regions of their DNA strands. These proteins facilitate the actual exchange of single strands of DNA between the participating DNA partners. The RecA protein of *Escherichia coli* is the prototypic member of this family of functionally and genetically similar proteins.

The co-protease activity is another unique property of RecA protein. It is a highly specific self-cleavage of proteins (LexA repressor, UmuD mutagenesis factor and lambdoid phage repressor proteins) that is dependent on both ATP and single-stranded DNA (ssDNA).

The third activity of RecA protein is its direct participation in the replication-dependent bypass of mutagenic lesions in DNA. This function requires binding of the processed mutagenic complex, $Umu(D')_2C$, to the RecA protein filament that has assembled at the site of the DNA lesion, thereby enabling DNA synthesis beyond the lesion.

Each of these processes is discussed in more detail below.

The structure of RecA protein

For RecA protein to function in these processes, it must assemble on ssDNA to form a nucleoprotein filament known as the presynaptic complex (6, 19). This filament is an adaptable structure, capable of performing three separate functions (Figure 1): homologous recombination (interaction with double-stranded DNA (dsDNA)), SOS induction (cleavage of the LexA repressor) and SOS mutagenesis (interaction with the processed $Umu(D')_2C$ complex).

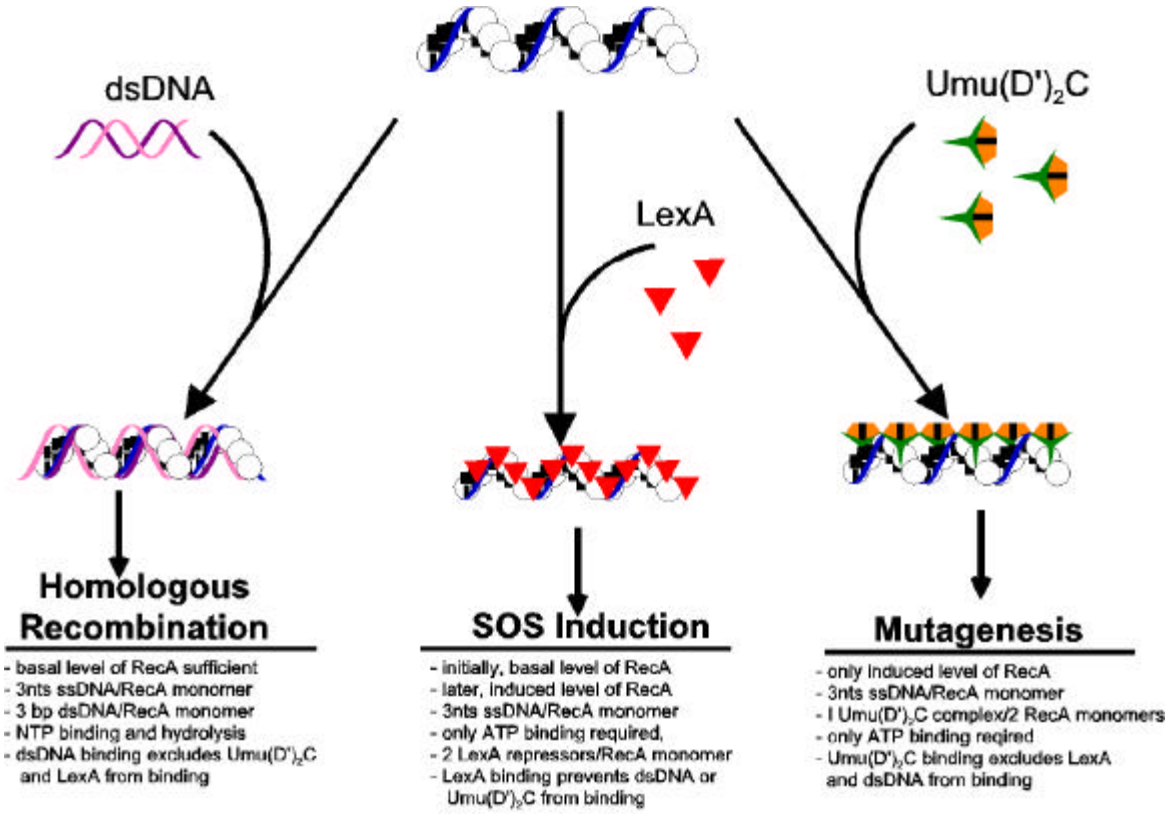

Figure 1. Roles of the RecA nucleoprotein filament in DNA metabolism. A schematic showing the three mutually exclusive functions of the RecA protein filament. The figure is adapted from Rehrauer, W. M., Bruck, I., Woodgate, R., Goodman, M. F., and Kowalczykowski, S. C. (1998), **submitted**.

The active nucleoprotein filament is a helical complex of RecA protein monomers wrapped around single-stranded DNA at a stoichiometry of 3 nucleotides/monomer and 6.2 monomers per turn (Figure 2A-D). Under certain conditions, RecA protein will also form filaments on dsDNA. Filament assembly is highly cooperative and occurs in the 5'→3' direction relative to the ssDNA to which it is bound. The resulting filament has a regular, right-handed, helical structure, and the prominent feature is a large helical groove (Figure 2A). One side of the groove is smooth, while the other is penetrated by the protrusion of the individual monomers (Figure 2B). This groove is the binding site for the LexA repressor (22) and is proposed to be involved in the binding of dsDNA. The binding of the LexA repressor and dsDNA to the nucleoprotein filament is competitive, indicating that they bind at the same, or overlapping site, on the filament.

The nucleoprotein filament can exist in two forms: active and inactive. The inactive filament is formed in the absence of a nucleoside triphosphate cofactor and exists in a collapsed conformation with a helical pitch of 65 . The binding of a nucleoside triphosphate (NTP) cofactor (typically ATP), converts RecA protein to a high-affinity ssDNA-binding state, which is the active conformation of RecA protein that is required for all the activities of the protein. This active form of the filament has an extended conformation with a helical pitch of 95 . It is this extended conformation that is the active species in RecA protein-promoted reactions.

Within the active form of the nucleoprotein filament, the conformation of both ss- and dsDNA differs significantly from that of B-form DNA (6). The bound DNA is extensively stretched (to approximately 150% the length of B-form) and, in the case of dsDNA, the helix is also unwound. Electron micrographic analysis (Figure 2C) and neutron scattering reveal that the DNA is located near the axis of the nucleoprotein filament.

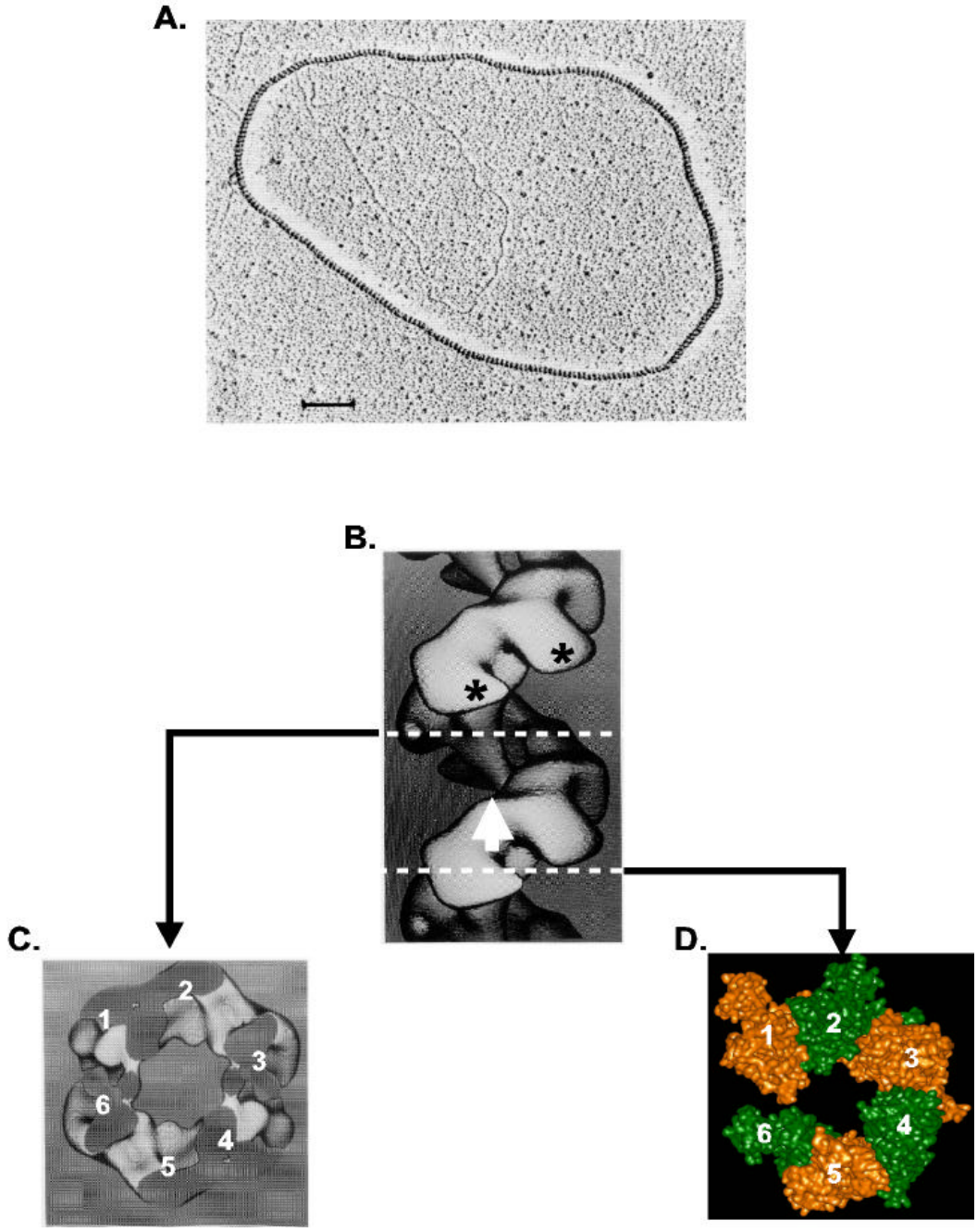

Figure 2. The nucleoprotein filament formed by RecA protein. (A), A filament formed on dsDNA with ATPγS as cofactor (Stasiak, A., and Egelman, E. H. (1988) in *Genetic Recombination* (Kucherlapati, R., and Smith, G. R., Eds.), American Society for Microbiology, Washington, D. C., pp 265-308). The striations in the filament are the groove which is discussed in the text. The bar in the figure represents 0.1 nm. (B), A close-up of the filament (the DNA is not visible) shown in A. The asterisks indicates the lobes of monomers that protrude into the groove and the white arrow indicates the smooth surface of the groove. C and D are cross-sections of the filament showing a single turn. The views shown in B and C, are three-dimensional re-constructions of electron micrographs of RecA filaments formed on dsDNA with ATPγS. The view in D, shows a single turn of the RecA filament as seen in the crystal structure (19). For this view, the individual monomers are coloured alternately in orange and yellow. The numbers in C and D, indicate the 6 monomers making up the turn in the cross-section; the DNA would be located in the central hole in each cross-section.

DNA strand exchange proteins isolated from a wide variety of organisms including bacteriophage, budding yeast, and humans (15), are very similar (Figure 3) and assemble into nearly identical nucleoprotein filament structures, highlighting the importance of the nucleoprotein filament in RecA protein function. Thus, the assembly of RecA protein onto ssDNA promotes formation of the nucleoprotein filament that is the central structure in RecA protein function. This unique structure (the filament) has the capacity to bind dsDNA and catalyse the exchange of single strands of duplex DNA; to bind and cleave repressors and proteins important to mutagenesis; and also, to bind and target the Umu(D')$_2$C complex to DNA lesions, thereby facilitating SOS mutagenesis.

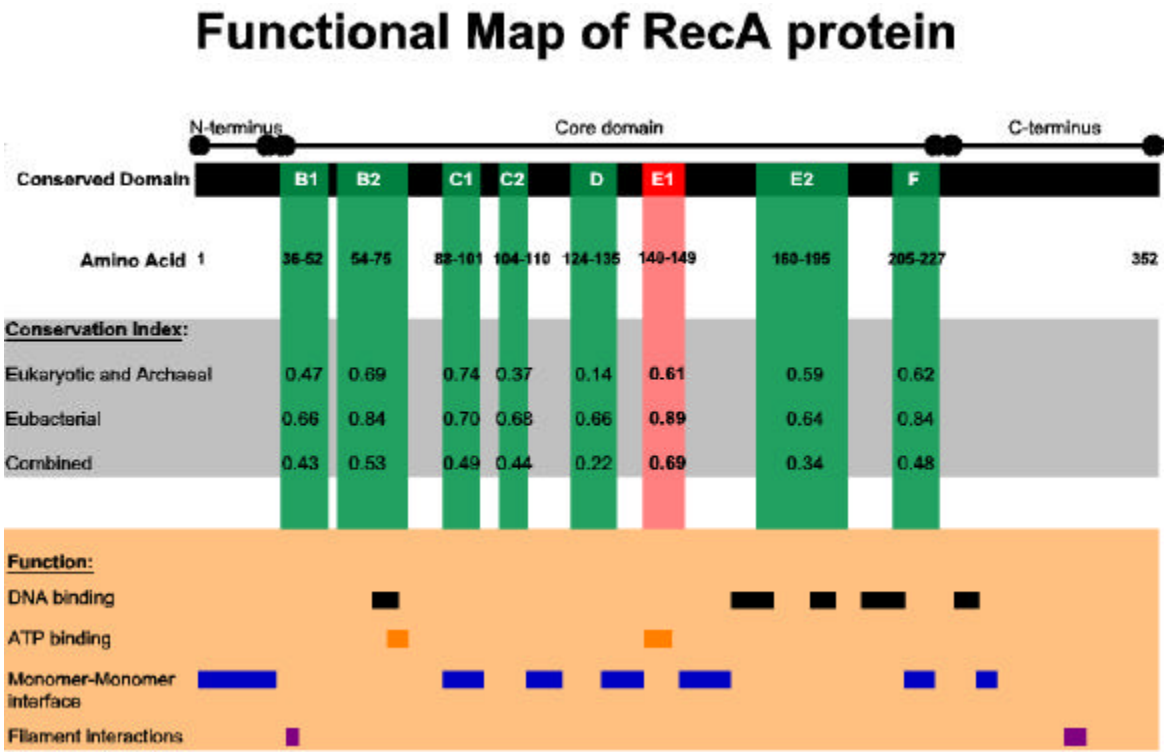

Figure 3. Functional map of the RecA protein. RecA protein is presented as a linear amino acid sequence. There are 8 highly conserved sub-domains found in all RecA protein homologues. These are highlighted in green and red, and the amino acids corresponding to each domain are shown at the top of each domain. The most conserved domain is sub-domain E1, shown in red. The Conservation Index (CI) for each sub-domain is shown in the grey box. A CI=1.00 indicates that this region is invariant in all RecA protein homologues. The combined index is a combined analysis of all RecA protein homologues and the number shown is not an average CI of the eubacterial, eukaryotic and archaeal conservation indices. The functional domains of RecA protein are highlighted in the peach box. The regions involved in DNA binding (black), ATP binding (orange), the Monomer-monomer (M-M) interface (blue) and filament-filament interactions (mauve) are displayed.

The role of RecA protein in DNA strand exchange

RecA protein promotes the exchange of single-strands of DNA between two homologous DNA molecules (3, 11, 18). The functional form of RecA protein in this reaction is the extended, presynaptic filament assembled on ssDNA. The nucleoprotein filament possesses two DNA binding sites: the primary site, which is required for assembly and is in contact with the ssDNA, and the secondary site, which is constituted only upon filament formation and is located outside of the central axis of the filament. The secondary site is responsible for binding both the incoming dsDNA and, following DNA strand exchange, the displaced ssDNA product of this reaction.

DNA strand exchange catalysed by RecA protein is the paradigm for homologous pairing processes *in vitro* (Figure 4). The most widely studied substrates are circular ssDNA

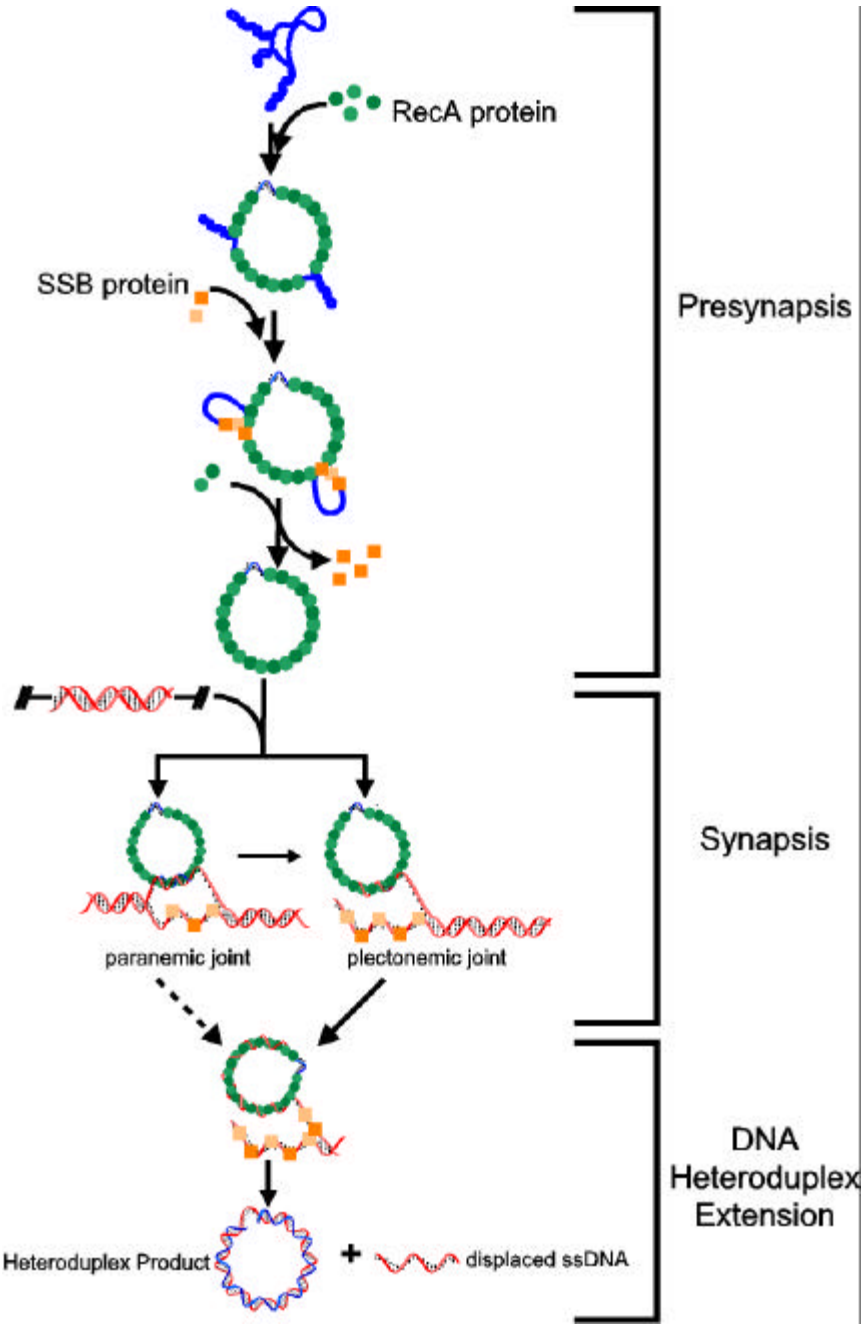

Figure 4. The DNA strand exchange reaction promoted by the RecA protein. RecA protein is represented as a sphere and SSB protein as a square. The three stages shown are: presynapsis, synapsis and DNA heteroduplex extension. Details are discussed in the text. This figure is adapted from Bianco, P. R., R. B. Tracy, and S. C. Kowalczykowski. (1998). DNA strand exchange proteins: A biochemical and physical comparison. *Front Biosci* **3:**D570-D603.

(isolated from ϕX174 or M13 phage) and linear dsDNA (usually the linearized, replicative forms of these phages). Many pairs of substrates can be used, provided that one of them is at least partially single-stranded. The products of the complete exchange of DNA stands are nicked, circular dsDNA and linear ssDNA.

DNA strand exchange can be divided into three experimentally distinguishable steps: (I) presynapsis, (II) synapsis, and (III) DNA heteroduplex extension (4) (Figure 4). In presynapsis, RecA protein assembles onto ssDNA to form the nucleoprotein species that is active in the homology search.

Synapsis is a complex step, conceptually composed of random non-homologous contacts occurring between the presynaptic complex and naked dsDNA; the search for DNA sequence homology; and base-pair switching, all resulting in the formation of a plectonemic, joint molecule intermediate. DNA heteroduplex extension then completes the reaction by migrating the nascent DNA heteroduplex joint in a polar fashion.

Presynapsis

In this stage of the reaction, RecA protein assembles in an ordered fashion onto ssDNA to produce the active nucleoprotein complex. Due to limitations imposed by DNA secondary structure, RecA protein is unable to form a contiguous nucleoprotein filament. The single-stranded DNA binding protein (SSB protein) of *E. coli*, is an accessory factor whose role is to remove the secondary structure (8); the SSB protein is then displaced by further binding of the RecA protein. This results in the formation of a complete nucleoprotein filament and the concomitant reconstitution of the second DNA binding site. The second DNA binding site exists only in the complete nucleoprotein filament, is vacant, and is used to bind a second DNA molecule (either ss- or dsDNA) (7).

Synapsis

During this stage of the reaction, dsDNA binds to the filament and, upon homologous recognition, a plectonemic joint molecule intermediate is formed (defined below). The second DNA molecule binds to the secondary DNA binding site of the nucleoprotein filament in a sequence-independent fashion.

Once bound, a search for homology takes place. The search is rapid, occurring within minutes, and requires that the binding of dsDNA to the secondary site be both weak and transient. The problem of the homology search is analogous to the problem that sequence-specific DNA binding proteins face in locating their target sequence. However, for RecA protein, the scale of the search problem is much larger: the binding protein is the entire nucleoprotein filament (which can consist of thousands of protein monomers, depending on the ssDNA length). Since the binding of RecA protein to ssDNA is largely non-specific, specificity in the search process is conferred by the sequence of the ssDNA within the filament. Although the entire nucleoprotein filament is involved in the search for homology, the minimum length of homology required for recognition is as low as 15 nucleotides *in vitro* (21), which is somewhat less than that needed *in vivo*, where homologous recombination requires minimally, about 23-40 bp of homology.

The recognition of homology takes place when the ssDNA within the presynaptic filament hydrogen bonds, presumably via non-Watson-Crick base pairing, to the bound dsDNA, in a mechanism that does not require stable triplex DNA formation (7). How then does RecA protein "sense" when homology has been located? During the homology search process, the dsDNA is topologically unwound, and is "tested" for complementarity with the ssDNA within the filament. It is the recognition of homology between these two DNAs that provides the signal to RecA protein that homology has been located and base switching occurs. This requires a local denaturation of the dsDNA molecule and the subsequent exchange of the identical single-strands of DNA. These steps may be simultaneous or separated in time, but the result is production of an intermediate known as a joint molecule. Immediately after base-pair switching, the heteroduplex dsDNA product occupies the primary site, whereas the displaced ssDNA occupies the secondary site (10).

Two types of joint molecules may form, depending on the topological constraints of the DNA: either paranemic or plectonemic (2). A paranemic joint is one in which the individual complementary strands do not intertwine, producing a molecule that is base-paired, but not topologically linked. A plectonemic joint is one in which the incoming single strand is intertwined around its complement, as in native dsDNA. In the reaction displayed in Figure 4, paranemic joints form at interior sites of duplex DNA, and plectonemic joints form at the ends of the duplex substrate. Since there are far more internal sites than end sites, paranemic joints are more likely to form and are thus likely intermediates on the reaction pathway to the formation of the more stable, plectonemic joint molecules.

DNA heteroduplex extension

Once a plectonemic joint has formed, the branch migration phase of DNA strand exchange commences (4). During this phase, the DNA heteroduplex in the nascent joint molecule is extended until complete exchange of DNA

strands occurs, resulting in a nicked, double-stranded circle. Though kinetically distinct, branch migration may not be a mechanistically separate step, but rather may represent a continuation of plectonemic joint molecule formation. Branch migration proceeds in a 5'→3' direction relative to the incoming single-strand (the same direction as RecA protein polymerisation (16)), at a rate of 2-10 bp sec^{-1}, requires ATP hydrolysis, and induces torsional stress in the dsDNA.

Energetics

RecA protein is a DNA-dependent NTPase with a single active site present in each monomer for the binding and hydrolysis of ATP and other nucleoside triphosphates (14, 17). The enzyme can to hydrolyse ATP with either ss- or dsDNA as cofactor. The active species in ATP hydrolysis is the nucleoprotein filament, with ATP being hydrolyzed uniformly throughout the filament and with no detectable enhancement at filament ends. Hydrolysis of ATP to ADP results in conformational changes in RecA protein that serves to modulate RecA between the "high-affinity" DNA-binding and "low-affinity" DNA-binding states, respectively.

Since ATP hydrolysis coincides with the pairing and exchange of DNA strands, it was initially thought that ATP hydrolysis was a requirement for DNA strand exchange. It is now known, however, that neither the hydrolysis of ATP nor the presence of a high-energy phosphate bond is necessary for DNA strand exchange (12). DNA strand exchange requires only that RecA protein assume the high-affinity ssDNA-binding state brought about by ATP binding. The binding of either the non-hydrolyzable analogue of ATP, ATPγS, or the non-covalent complex of ADP-AlF_4^-, also induces the high-affinity DNA-binding state of RecA protein and supports DNA strand exchange. Although the hydrolysis of ATP is not required for the exchange of DNA strands, it is needed to dissociate RecA protein from the heteroduplex products of DNA strand exchange once the reaction is complete, to facilitate the bypass of structural barriers such as heterologous sequences, and to maintain the polarity of DNA strand exchange.

In addition to ATP, other NTP cofactors also support DNA strand exchange. The key factor in determining whether an NTP can function as a cofactor, is its ability to stabilise the high affinity ssDNA-binding state of RecA protein. This ability is directly related to the $S_{0.5}$ for that cofactor (where $S_{0.5}$ is the substrate concentration required for half-maximal ATPase activity). Only those nucleoside triphosphate cofactors whose $S_{0.5}$ value is 100 - 120 μM or lower, are capable of stabilising the DNA strand exchange-active conformation of RecA protein. Thus in addition to ATP, either dATP, purine riboside triphosphate, or UTP can function as cofactors for DNA strand exchange *in vitro*. Of these, ATP, dATP, and UTP are likely cofactors for RecA protein-promoted processes *in vivo*.

The regulatory role of RecA protein

The SOS regulon is a group of approximately 20 unlinked genes (Table I) whose expression is induced to high levels following exposure to DNA damaging agents. The enzymes encoded by these induced genes, function to excise DNA damage and to facilitate the error-prone, recombinational repair of DNA . RecA protein plays a key role as a regulator of the SOS regulon and inducible DNA repair, where it functions as a co-protease, stimulating the auto-catalytic cleavage of a number of proteins (9, 14, 17).

The primary controller of the expression of the genes in the SOS regulon is the LexA repressor, which binds to the SOS box of these SOS-inducible genes and limits their transcription. After a DNA-damaging event such as UV-irradiation, the co-protease activity of RecA protein becomes “activated”. Activation occurs due to the generation of ssDNA resulting either from the action of nucleases or from stalled replication forks. The ssDNA is bound by RecA protein in the presence of a nucleoside triphosphate cofactor, promoting nucleoprotein filament formation that leads to cleavage of the LexA repressor and induction of SOS genes, including *recA* (1). Genes with operators which bind LexA protein weakly are the first to be expressed fully (*e.g.*, *recA*). If the damage persists, or if sufficiently high quantities of the damaging agent are used, the concentration of activated RecA protein increases, leading to further cleavage of LexA protein, and to de-repression of even those genes whose operators bind LexA tightly (*e.g. sulA*).

Under normal cellular conditions, *recA* expression is repressed and the basal level of RecA protein is maintained at ~1000 molecules per cell. Following LexA repressor cleavage, the level of RecA protein in the cell increases by as

Table I. Genes of the SOS regulon.

Gene	Position (min)	Gene Product Function
lexA	91.6	Represses transcription of ~ 20 genes by binding to operators
recA	60.8	DNA strand exchange; induces SOS; activates UmuD; SOS mutagenesis
recN	59.1	Unknown
recQ	86.2	DNA-dependent ATPase and helicase; can both promote homologous recombination and disrupt illegitimate recombination
sulA (sfiA)	22.0	Inhibits cell division
umuD	26.5	Active in SOS mutagenesis as cleaved UmuD'; forms complex with UmuC
umuC	26.5	Forms a complex with UmuD and Umu(D')$_2$
uvrA	91.9	Excision-repair nuclease subunit A; part of the UvrABC endonuclease that initiates excision repair
uvrB	17.5	Helicase and ATPase; excision-repair nuclease subunit B; part of the UvrABC endonuclease that initiates excision repair
uvrC	43.0	Excision-repair nuclease subunit C; part of the UvrABC endonuclease that initiates excision repair
uvrD	86.1	DNA-dependent ATPase and helicase required for excision repair
ruvA	41.9	Forms a complex with RuvB; recognizes Holliday junctions
ruvB	41.9	Forms a complex with RuvA; branch migration helicase
ssb	92.0	Binds to ssDNA
dnaN	83.6	DNA synthesis as part of DNA polymerase III
dnaQ	5.1	DNA synthesis as part of DNA polymerase III
dinF	91.7	Unknown
dinG	18.0	Unknown

much as 20-fold. The increase in the level of RecA protein is rapid, occurring at a rate of 10 molecules per second, and reaches a maximum within one hour of a DNA damaging event. RecA protein levels return to the basal level within 4 to 6 hrs following the initial damage event. This reduction is presumably due to removal of the inducing signal *via* recombinational repair of the DNA damage eliminating the agent that activated RecA protein. As a result, the intracellular concentration of LexA repressor increases, since RecA protein is no longer able to induce its cleavage. This re-establishes repression of the SOS system and returns the cell to its uninduced state.

The role of RecA protein in mutagenic bypass

When an unrepairable DNA lesion is encountered by DNA polymerase III, replication is stalled until an error-prone lesion bypass occurs. For this to occur, a special, or modified error-prone, DNA polymerase is required to

insert an incorrect nucleotide opposite the lesion (producing a mutation) and then allowing normal DNA synthesis. This process is referred to as SOS mutagenesis or error-prone repair, and is the last recourse to ensure cell survival when all other error-free processes have acted (20).

SOS mutagenesis requires high levels of functional *umuC* and *umuD* gene products. In exponentially growing *E. coli*, these proteins are expressed at a low, basal level (due to LexA binding to the SOS box of the *umuDC* operon) with approximately 180 UmuD proteins per cell and an undetectable amount of UmuC protein. Following DNA damage, the levels of RecA protein increase and this results in (a), the cleavage of the LexA repressor causing de-repression of expression of the *umuDC* operon leading to an increase in the levels of UmuC protein to 200 molecules per cell and UmuD protein to 2400 copies per cell and (b), the proteolytic processing of UmuD to UmuD' by the activated RecA protein (5, 13). This processing yields two fragments of UmuD protein: a small N-terminal fragment and a larger, C-terminal fragment. It is the C-terminal fragment (referred to as UmuD') that is the active species in UV-induced mutagenesis (13); two UmuD' molecules associate with UmuC, forming the $Umu(D')_2C$ complex. These complexes bind to and are targeted by the activated RecA nucleoprotein filament to DNA lesions where they are proposed to function as a specialised elongation factor to interact with a stalled DNA polymerase III helping it to resume DNA synthesis across the lesion.

Summary

RecA protein is an essential component of homologous recombination and DNA repair in *E. coli*. The key, universally conserved structure, in each of these processes, is the RecA nucleoprotein filament. It is a ternary complex consisting of RecA protein, ssDNA and a nucleoside triphosphate cofactor. This structure has the capacity to bind, in a mutually exclusive manner, dsDNA, the LexA repressor and the $Umu(D')_2C$ complex. Binding of dsDNA to the filament allows RecA protein to function as a DNA strand exchange protein. The binding of the LexA repressor to the filament induces an auto-catalytic cleavage which inactivates the repressor which, in turn, results in de-repression of the SOS regulon. Finally, the binding of the processed $Umu(D')_2C$ complex to the filament switches the role of RecA protein from a recombination one to a mutagenic one. The *in vitro* capabilities of this specialised nucleoprotein filament are consistent with the roles of RecA protein *in vivo*, and explain the pleiotropic effects of mutations in the *recA* gene.

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Glossary

1. SOS response – a DNA repair system induced by DNA damage

2. SOS Mutagenesis – an error-prone DNA repair mechanism that occurs under conditions of extreme DNA damage

3. DNA Strand Exchange – a process whereby strands of DNA are exchanged between two homologous DNA molecules

4. Nucleoprotein filament – a complex of RecA protein, DNA and a nucleoside triphosphate cofactor

5. Co-protease – a specific activity of the RecA nucleoprotein filament which allows it to bind to a protein and cause that protein to cleave itself.

6. Plectonemic joint molecule – a DNA strand exchange intermediate where the complementary strands of DNA are both base paired and intertwined

7. Paranemic joint molecule – a DNA strand exchange intermediate where the individual complementary strands of DNA are base paired but not intertwined

8. $S_{0.5}$ – the concentration of a substrate required to produce half-maximal activity