Table 6 Use and Sensitivity of Direct and Indirect Autoradiography, and Fluorography

Isotope	Method	Example Applications	Preflashed Film	Intensifying Screen	Temperature of Exposure	Approximate Detection Limit After Exposure for 24 h (dpm/cm^2)
^{3}H	Direct	Whenever very high resolution is required (e.g., in situ hybridization)	No	No	Room temp.	8×10^6
	Fluorography	Acrylamide gels	Yes	No	−70°C	8×10^3
^{14}C, ^{35}S	direct	DNA sequencing	No	No	Room temp.	6×10^3
or ^{33}P	indirect	SDS-polyacrylamide gels	Yes	Yes	−70°C	4×10^2
^{32}P	direct	DNA sequencing	No	No	Room temp.	5×10^2
	indirect	Southern analysis	Yes	Yes	−70°C	5×10^1
^{125}I	direct	Subcellular localization	No	No	Room temp.	2×10^3
	indirect	Western blotting	Yes	Yes	−70°C	1×10^2

autoradiography and fluorography (including use of intensifying screens).

As discussed in Section 2, alternatives to radioisotopes should be used whenever possible. Nonradioactive labeling methods for DNA and proteins are improving all the time. Light-based detection techniques seem to be particularly successful in western blotting, for example. In the case of ^{32}P, a general rule at present is that if acceptable results are obtained within 24 hours exposure to autoradiography, a nonradioactive alternative should be sought. If longer exposures are required, particularly with intensifying screens, it is likely that ^{32}P is still required to meet the necessary sensitivity.

6.5 Personal Dosimeters

Personal radiation monitors are either film badges or thermoluminescent detectors (TLDs). The latter contain a phosphor such as LiF that becomes and remains excited upon exposure to radiation. Heat treatment results in light emission, the intensity of which relates to dose received. For hand dosimetry, small TLDs can be worn on the fingers, under gloves.

Personal dosimeters are not appropriate for workers using low energy emitters such as ^{14}C, ^{35}S, or ^{3}H, but they are of value to users of ^{32}P or ^{125}I. Their issue is at the advice of senior staff with appropriate training and responsibility for radiation protection, following dosimeter calculations and estimates of exposure levels to individuals.

See also Labeling, Biophysical.

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Raman Spectroscopy in Protein Analysis: *see* Protein Analysis by Raman Spectroscopy.

RecA Protein, Structure and Function of

Michael M. Cox

1 Overview
- 1.1 Homologous Genetic Recombination
- 1.2 Biological Function of RecA

2 Structure
- 2.1 Primary Structure
- 2.2 Three-Dimensional Structure

3 DNA Strand Exchange as a Model for Recombination and Recombinational DNA Repair
- 3.1 Fundamentals
- 3.2 DNA Binding and Filament Formation
- 3.3 DNA Pairing
- 3.4 ATP Hydrolysis
- 3.5 Models

4 RecA Protein as a Coprotease

5 Other Recombination Proteins
- 5.1 Proteins Involved in the Initiation of Recombination
- 5.2 Proteins That Act in the Later Stages of Recombination

Key Words

Genetic Recombination A rearrangement of genetic information within or between nucleic acids.

number of disintegrations in the sample, and the strength of the signal relates to the energy of the absorbed radiation.

Scintillation probes are small handheld instruments useful for monitoring γ radiation, say from ^{125}I. They display either cps values or for, dosimetry purposes, microsieverts per hour. One of these instruments should be available whenever ^{125}I is used. As with ionization monitors, the counters should be calibrated officially once a year and can be roughly calibrated by the user as an aid to monitoring (see Section 6.1).

Bench scintillation counters are designed for analysis of large numbers of experimental samples. They either contain solid scintillators for detection of X-, or γ-radiation or are designed for liquid scintillation counting. The latter instruments are found in most biochemical laboratories and are designed particularly for detecting β emitters. The sample, usually in liquid form, is pipetted into a small container (or vial) to which a scintillation fluid is added.

The many recipes for scintillation fluids (often referred to as cocktails) are based on an organic solvent and one or more solutes termed primary fluors (when one solute is used) or primary and secondary fluors (when two solutes are used). These fluors enhance the light output and shift the wavelength to the optimum for photomultiplier tubes. Most laboratories buy their cocktails ready prepared, and the manufacturer's catalog should be carefully consulted. Essentially there are three forms of cocktail: a relatively straightforward formulation for counting organic samples, detergent-containing fluids for counting aqueous samples, and fluids that form gels for counting dispersed powders. The most commonly used ingredients are toluene or xylene as solvent, PPO (2,5-diphenyloxazole) or butyl-PBD [2-(4-*t*-butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiazole] as primary solute, POPOP [1,4-bis(5-phenyloxazol-2-yl)benzene] as secondary solute, and Triton-X100 as detergent.

An important concept in liquid scintillation counting is quench. This is anything that reduces counting efficiency—for example, colored compounds or chemicals such as chloroform that interfere with the scintillation process. Because a range of experimental samples may be subject to differing degrees of quench, it is necessary to calculate the counting efficiency of every sample individually. This can be done by recounting samples following addition of known amounts of radioactivity (an internal standard). Since, however, this procedure is tedious for a large number of samples and contributes to the problem of waste disposal, instruments have built-in means for automatically determining the counting efficiency. Specifically, there is built into the machine a means of counting a solid source of radioactivity that irradiates the sample vial. When quenching occurs, the energy spectrum of the radiation, as detected by the photomultipliers, appears to shift to lower energy values. Analysis of this shift results in an assessment of counting efficiency within the sample vial. The instrument then corrects for the efficiency and prints out data as dpm. Although this system works well in most cases, it is essential to realize that it can be relied on only when the sample is in solution in the vial; it is not appropriate for heterologous systems such as counting filter papers placed in scintillation fluid.

The ability of scintillation counters to analyze the energy of radiations is of great value. Radioisotopes have characteristic energy spectra. If these are sufficiently different, a scintillation counter can detect radioisotopes separately, even when they are present in the same vial. All β spectra are similar in shape, but different radioisotopes have differing maximum energies (see Table 3); thus ^{3}H, ^{14}C, and ^{32}P, for example, can be distinguished by their energy spectra. This means that dual labeling experiments with these radionuclides are feasible, and scintillation counters can be used to determine types of contamination. The only way to monitor for ^{3}H is to perform wipe tests with damp tissues and count in a liquid scintillation counter; if other forms of contamination were present, the anomaly would be detected by the counter on the basis of spectral analysis.

Liquid scintillation counting sometimes is accompanied by light output from scintillation fluid that is not dependent on radioactivity. That is, the counts per minute exceed the true dpm. When this phenomenon is caused by storage of scintillation fluid in bright sunlight, it is known as photoluminescence; when it is due to the presence of certain chemicals, it is called chemiluminescence. Most modern counters are able to detect such unwanted luminescence and make a note to that effect on the printout. In doubtful cases, samples can be stored in the dark for a few hours, then recounted.

6.3 Cerenkov Counting

High energy β particles travel through water faster than light, causing along their path polarization of molecules that emit photons of light as they return to a ground state. The energy of radiation from ^{32}P is high enough to cause the Cerenkov effect. Consequently water can be used in place of scintillation fluid for this radioisotope. The counting efficiency is relatively low (≈30% compared with >90% in a scintillation cocktail), and the counter should be specifically calibrated for it, although an acceptable short-cut is to count the samples as if they were ^{3}H. The big advantages of Cerenkov counting are that aqueous samples can be counted and recovered for experimental use and organic radioactive waste does not accumulate.

6.4 Autoradiography

Many experiments in molecular biology rely on autoradiography, in particular, analysis of nucleic acid samples separated on gels (Southern blotting, northern blotting, DNA sequencing) or detection of particular clones or plaques by hybridization screening. The basic principle underlying autoradiography is that light, a β particle, or an X- or γ-ray can produce silver atoms in crystals of silver halide present in photographic emulsions. This process is reversible if only a single atom of silver, produced from one photon, forms in a crystal. However, silver atoms catalyze the conversion of silver halide to silver by the chemicals in the developer; this stabilizes the image.

If the radiation is of very low energy (e.g., from ^{3}H), it cannot penetrate very far and would, for example, be absorbed by the matrix of an agarose or polyacrylamide gel. Conversely, radiation that is very penetrating (e.g., from ^{32}P or ^{125}I) passes through film without giving up much of its energy and is therefore inefficiently detected. To combat these difficulties, investigators use the techniques of fluorography, in which samples such as gels are impregnated with fluors such as PPO (see Section 6.2), and indirect autoradiography, in which intensifying screens partly absorb penetrating radiation and emit light. These adaptations increase sensitivity but reduce resolution. In both these cases exposure at low temperature is required to stabilize the latent image. In addition, a brief (<1 ms) flash of light is required to preexpose the film and make it more responsive to low energy signals. Specific films are sold for direct and indirect autoradiography. Table 6 summarizes some of the key features of

Heteroduplex DNA Double-stranded DNA containing complementary strands derived from different and largely homologous DNA molecules via recombination, and including base pair mismatches or other DNA lesions.

Heterologous Sequences Nucleic acid sequences that are dissimilar and evolutionarily unrelated.

Holliday Intermediate A DNA structure in which two DNA molecules are linked by the breakage, crossover, and rejoining of one strand from each.

Homologous Sequences Nucleic acid sequences that are similar or identical by virtue of an evolutionary relationship.

Hybrid DNA Double-stranded DNA containing complementary strands derived from different homologous DNA molecules via recombination.

SOS A regulated response to DNA damage and other cellular traumas in bacteria.

Triplex A nucleic acid structure in which three nucleic acid strands are interwound and hydrogen-bonded.

The RecA protein is a central component of the bacterial system that mediates recombinational DNA repair, chromosomal partitioning at cell division, and homologous genetic recombination. It also plays a key role in the regulation of the SOS response to DNA damage. RecA protein forms long, helical nucleoprotein filaments on the DNA. These filaments are the active species in the DNA pairing and strand exchange reactions in recombinational processes and in the induction of SOS. The continuing study of RecA protein offers insights into bioenergetics, DNA structure and topology, and protein–DNA interaction, as well as a broader understanding of DNA metabolism.

1 OVERVIEW

The product of the *recA* gene is a multifunctional protein that occupies an important crossroads in bacterial DNA metabolism. The *recA* gene was first identified in 1965 by A. J. Clark and A. Margulies. RecA protein plays a central role in recombinational DNA repair, chromosomal partitioning at cell division, and genetic recombination. It also regulates a global cellular response to DNA damage. Finally, it facilitates replicative bypass of DNA lesions when DNA damage is extensive. Biochemical interest is focused on the mechanism(s) by which RecA protein mediates these varied functions.

1.1 Homologous Genetic Recombination

For more than a century, geneticists have made use of the naturally occurring exchanges of genetic information between homologous chromosomes to study heredity and map genes. The molecular process underlying these genetic exchanges is known as homologous genetic recombination.

A genertic model illustrating the kinds of molecular process that must occur during recombination is presented in Figure 1. A key requirement is the alignment of homologous sequences in two DNA molecules. DNA strands in both DNA molecules must be broken (either before or after alignment) to initiate a strand exchange.

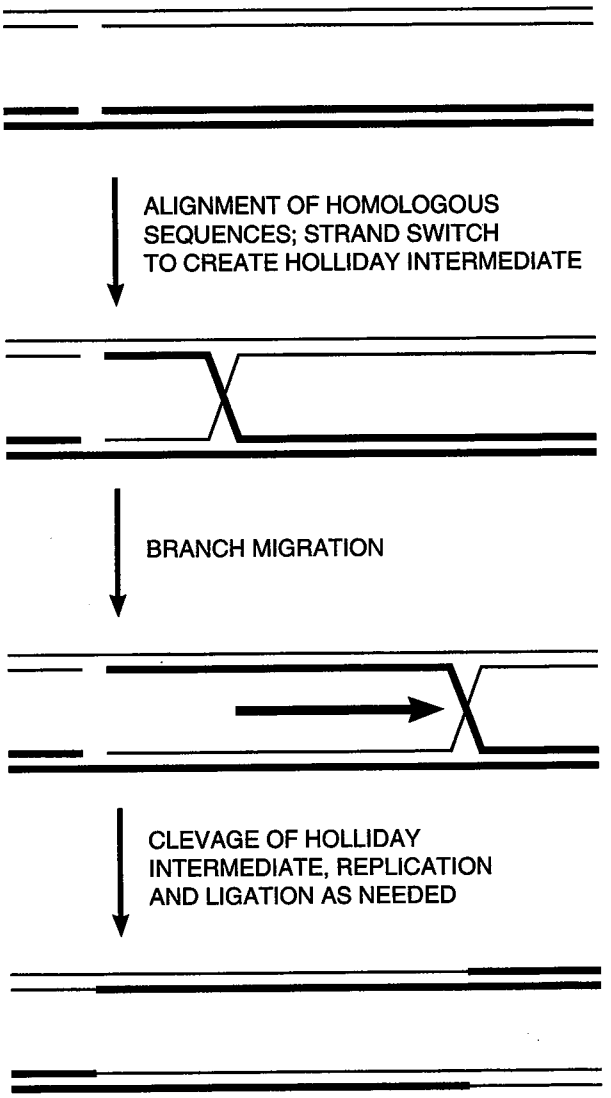


Figure 1. One model for homologous genetic recombination between two homologous chromosomes (thick and thin lines). Regions of the DNA intermediates and products in which thick and thin lines are paired are called heteroduplex DNA. There are many variants of this model; this depiction shows the kinds of process that must occur to bring about recombination.

This results in formation of a Holliday intermediate, a structure named for Robin Holliday, who first proposed it in 1964. The heteroduplex DNA formed in this exchange is then extended by branch migration. Finally, the Holliday junction is cleaved and the ends are repaired.

The first genes with products involved in recombination were identified in *E. coli* in 1965 by A. J. Clark. Recombination genes now include *recA, recB, recC, recD, recF, recG, recJ, recN, recO, recQ, recR, ruvA, ruvB, ruvC,* and *ssb.* Mutations in the *recA* gene exhibit the most complex phenotypes. The product of this gene is a multifunctional protein called the RecA protein. It is generally believed to play a major role in all steps of recombination that do not involve covalent bond cleavage or formation, including the pairing DNA molecules and the exchange of DNA strands between them.

1.2 Biological Function of RecA

As suggested by the number of genes involved and the multitude of complex patterns of interaction between them identified by current research, the cell makes a substantial investment in homologous recombination pathways. The immediate selective value of RecA protein to the bacterial cell probably lies in at least two functions: recombinational DNA repair and chromosomal partitioning.

A bacterial cell growing in an aerobic environment is subjected to thousands of DNA lesions each cell generation, many of which are potentially lethal. Most of the damage is inflicted by highly reactive forms of oxygen. Accurate DNA repair is made possible to a large extent because DNA is double stranded. A lesion in one strand can be repaired by removing a segment containing the lesion, then using the undamaged strand as a template to guide synthesis of a replacement segment. When both strands are damaged (e.g., a double-strand break or cross-link) or when the lesion is in a single-stranded region of DNA, the information for accurate repair must come from another homologous DNA molecule via recombination. The prototypical phenotype of *recA* mutant cells is an extreme sensitivity to DNA damaging agents.

Homologous genetic recombination and recombinational DNA repair are closely related processes, and RecA protein plays a central role in both. Recombination, even when repair is not the object, has the important result of increasing the genetic diversity, hence viability, of a species. Cells lacking RecA function exhibit reductions in the efficiency of homologous recombination by factors of up to 5×10^4.

Cells with *recA* mutations exhibit a severe defect in chromosomal partitioning. This is evidenced in part by an abnormal number of chromosomes in many cells. About 10% of *recA* mutant cells contain no DNA at all.

RecA protein also has a third, and quite different function. It facilitates the autocatalytic cleavage of the LexA protein and related repressors (an activity often called the coprotease function) to permit expression of the SOS response to heavy DNA damage and other traumas. As a part of the SOS response, RecA has a role in a special replication pathway that permits bypass of DNA damage. Its biochemical function in the replication pathway is not yet understood. The strand exchange and coprotease activities provide a rare example of the combination of at least two very distinct biochemical functions (DNA pairing and coprotease) in one relatively small protein.

Current studies with RecA protein are focused on a variety of fascinating questions concerning protein–DNA interactions, DNA pairing intermediates that may represent novel DNA structures, bioenergetics, and the functional relationship between RecA protein and other recombination proteins.

2 STRUCTURE

2.1 Primary Structure

In *E coli,* the RecA polypeptide chain contains 352 amino acids with a combined molecular weight of 37,842. The protein occurs in virtually all bacteria, and the *recA* gene has been sequenced in more than 60 bacterial species. The primary structure is illustrated in Figure 2, with 56 invariant amino acid residues highlighted. The close relationship between bacterial RecA proteins is reinforced by sequence similarities at many other positions. There is available a wide range of *recA* mutants, and these have facilitated an understanding of structure–function relationships in this polypeptide.

The wide distribution of bacterial *recA* genes indicates that the protein evolved more than 1.5 billion years ago. The UvsX protein of bacteriophage T4 is a functional analogue of RecA protein with more limited sequence similarity. A RecA analogue has recently been identified in Archaebacteria. The protein, called RadA, is 23% identical to *E. coli* RecA protein.

Eukaryotic genes with significant sequence homology to *recA* have been found. In yeast, two such proteins, Rad51 and Dmc1 (26 and 24% identical to RecA, respectively), are important in genetic recombination. Both yeast proteins exhibit a stronger identity (40%) to the archaeal protein RadA. The Rad51 protein has been found in a wide variety of eukaryotes, including plants and humans.

2.2 Three-Dimensional Structure

The structure of RecA protein has been determined at 2.3 Å resolution by R. Story and T. Steitz. There is a major central domain flanked by two smaller subdomains at the N- and C-termini.

AIDENKQKAL	AAALGQIEKQ	F**G**KGSIMRLG	EDRSMDVETI 40
STGSLSLDI**A**	L**G**A**GG**L**P**MG**R**	IV**E**IY**G**P**ES**S	**GKTT**LTLQVI 80
AAA**Q**REGKTC	AFI**D**AEH**A**L**D**	PIYARKL**G**VD	IDN**L**LCSQ**P**D 120
T**GE**QA**L**E**I**CD	ALARSGAVDV	IVV**DSVAAL**T	**P**KA**E**IE**G**EIG 160
DSHMGLA**AR**M	M**S**Q**A**M**R**KLAG	NLKQSNTLLI	**F**INQI**R**M**K**I**G** 200
VMFGN**PE**T**T**T	**G**GN**ALKF**YAS	V**R**LDI**R**RIGA	VKEGENVVGS 240
ETRVKVV**K**N**K**	IAA**P**FKQAEF	QILYGE**G**INF	YGELVDLGVK 280
EKLIEKA**G**AW	YSYKGEKIG**Q**	**G**KANATAWLK	DNPETAKEIE 320
KKVRELLLSN	PNSTPDFSVD	DSEGVAETNE	DF

Figure 2. The amino acid sequence of the RecA protein of *E. coli.* The residues shown in bold are invariant among the more than 60 sequenced bacterial *recA* genes.

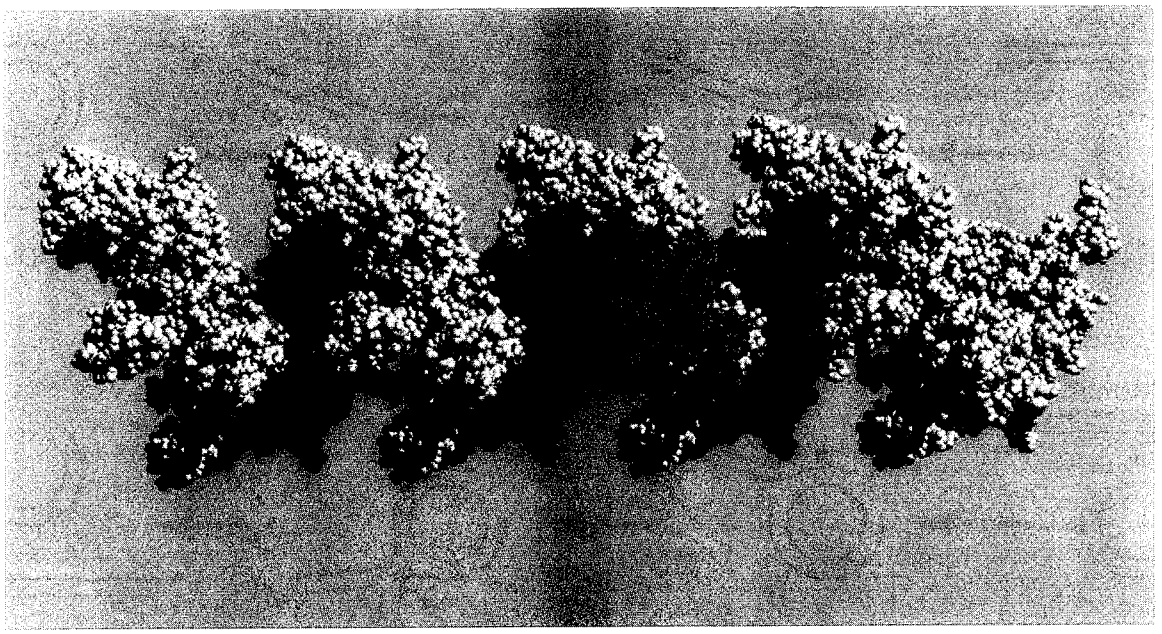


Figure 3. A representation of a segment of a helical filament formed by 24 monomers of RecA protein. Twelve monomers (two helical turns) are colored. The illustration is based on the structure determined by Story and Steitz and developed from the coordinates deposited in the Brookhaven Protein Data Bank, using the MidasPlus software obtained from the Computer Graphics Laboratory at the University of California, San Francisco. (See color plate 2.)

Monomers in the crystal are packed to form a continuous spiral filament, with six monomers per right-handed helical turn (Figure 3: see color plate 2). These results are consistent with other studies showing that RecA protein forms a helical nucleoprotein filament on DNA. The filament exhibits a deep helical groove. A variety of physical studies indicate that the groove can accommodate up to three DNA strands.

The central domain contains a single binding site for ATP or ADP. In the crystal structure, amino acid residues 66–73 make up the A-site nucleotide-binding motif of RecA. Additional contacts with bound nucleotides occur at Glu 96, Asp 100, Tyr 103, residues 140–144 (sometimes called a B-site motif), and at Gly 265. Most of these residues are either conserved or limited to conservative substitutions.

3 DNA STRAND EXCHANGE AS A MODEL FOR RECOMBINATION AND RECOMBINATIONAL DNA REPAIR

Typical DNA strand exchange reactions used to study RecA function in vitro are illustrated in Figure 4. The reactions shown are designed for convenience, since it is easy to distinguish products from substrates with a wide range of assays. DNA substrates are usually derived from bacteriophages, often M13 or its derivatives. The reaction can involve either three or four DNA strands, as shown, and it nicely mimics several of the putative steps in homologous genetic recombination (compare Figures 1 and 4). A Holliday intermediate is formed transiently in the four-strand reaction.

3.1 Fundamentals

There are at least four experimentally distinguishable phases of a RecA protein-mediated DNA strand exchange reaction. The active species is a RecA nucleoprotein filament that completely coats the single-stranded or gapped DNA substrate as the first phase in the reaction. In the discussion of Section 3.3, this DNA substrate is called DNA1. RecA binds to DNA1 without regard to its sequence. The resulting nucleoprotein filament, in contrast, is effectively a sequence-specific DNA-binding entity, with the sequence specificity determined by the bound DNA. The bound single strand is aligned with a linear duplex DNA (DNA2) in the second phase of the reaction. A novel DNA triplex may be an intermediate in the alignment process. In the third phase, there is a rapid strand switch to create 1000 bp or more of hybrid DNA. This produces the branched molecules shown in Figure 4. Phase 3 requires ATP but not its hydrolysis. The limited strand exchange in phase 3 can occur at either end of DNA2, and it cannot bypass structural barriers in the DNA such as short heterologous insertions in DNA2. In the fourth phase, the nascent hybrid DNA is extended in a facilitated branch migration reaction that proceeds until products are formed. This final phase is coupled to ATP hydrolysis. The branch migration is unidirectional ($5' \rightarrow 3'$ with respect to the single strand initially bound) and proceeds at a rate of 6 base pairs per second at 37°C. Phase 4 proceeds readily past mismatches, lesions, and even heterologous inserts (up to a few hundred base pairs in length) in one or both DNA substrates, a capability that is critical to its function in recombinational DNA repair.

RecA is a DNA-dependent ATPase, and ATP is hydrolyzed during strand exchange with a monomer k_{cat} of 20–22 min^{-1}. The apparent efficiency of the strand exchange reaction is low, with about 100 ATPs hydrolyzed for every base pair of hybrid DNA formed under typical reaction conditions. In the context of an isoenergetic DNA strand exchange between homologous DNA molecules, the consumption of chemical energy is excessive. The excess is not unusual, however, when considered in the context of DNA repair. The standard pathway for methyl-directed mismatch repair in *E. coli,* for

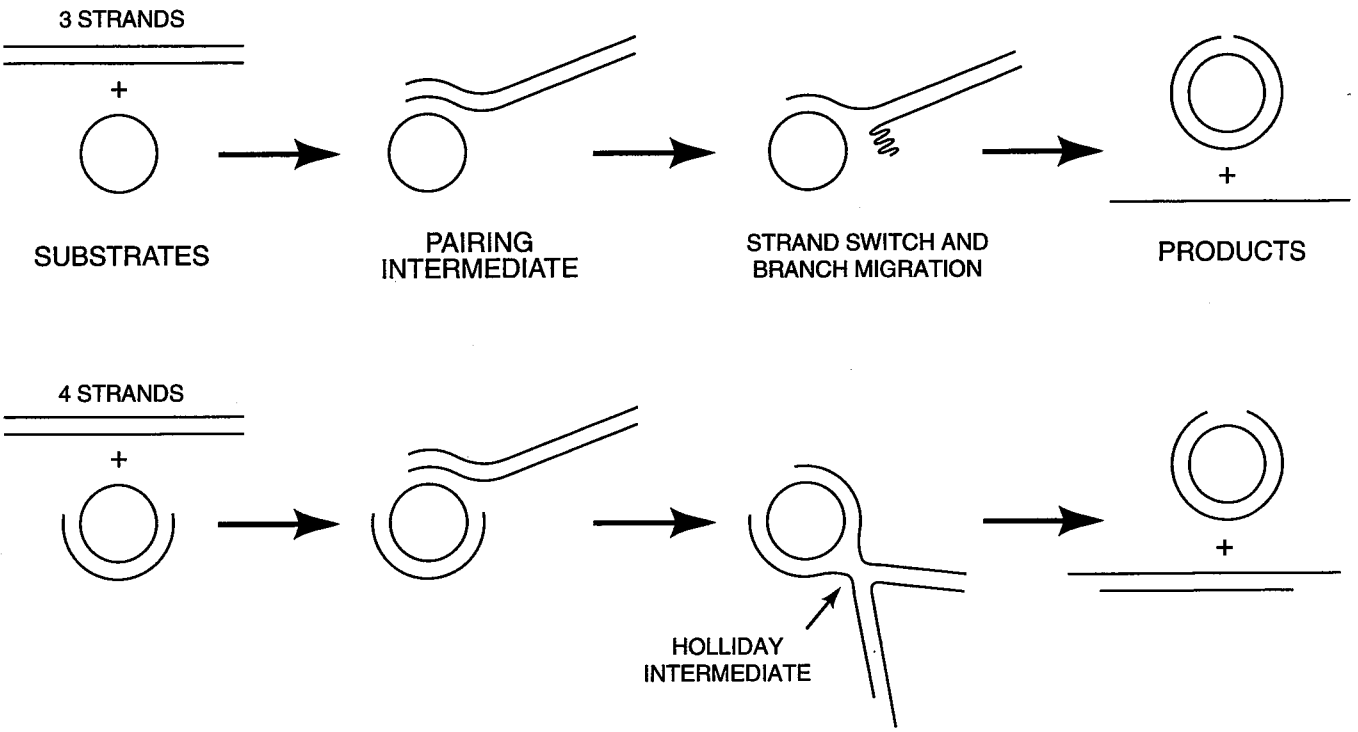


Figure 4. DNA strand exchange reactions promoted by RecA protein in vitro. The thin lines represent individual DNA strands.

example, can involve the degradation and replacement of a DNA strand 1000 or more nucleotides in length to repair one mismatch. As outlined in Section 3.4, ATP hydrolysis confers properties on the RecA-mediated DNA strand exchange reaction that are particularly important in recombinational DNA repair.

3.2 DNA Binding and Filament Formation

The filament has a right-handed helical form, with six RecA monomers per turn of the helix. One RecA monomer binds to three bases or base pairs. The bound DNA is extended and underwound, so that the bases are 5.1 Å apart and there are 18 base pairs per turn instead of the usual 10.5. The DNA is bound along the phosphate–deoxyribose backbone.

RecA protein binds rapidly to single-stranded DNA. Following nucleation, the filament is extended in the 5′-to-3′ direction along the DNA at rates exceeding 1000 monomers per minute. This unique direction of assembly is reflected in an inherent structural polarity exhibited by the assembled filament. If the substrate is a duplex with a single-strand gap, the nucleation occurs in the gap and the filament is extended $5' \rightarrow 3'$ along this strand to rapidly encompass the adjacent duplex DNA. ATP is hydrolyzed by the assembled nucleoprotein filament, but neither ATP binding nor hydrolysis is required for RecA protein binding to single-stranded DNA. The monomer k_{cat} for ATP hydrolysis for RecA bound to single-stranded DNA is 30 min^{-1}. In the absence of ATP or other nucleotide cofactor, a filament is formed which does not have the ordered structure of filaments formed in the presence of ATP, and the DNA within the filament is not extended. A similar "collapsed" filament is formed in the presence of ADP.

Binding is much slower on double-stranded DNA at neutral pH. The rate-limiting step is nucleation. Extension of nucleated filaments is rapid. DNA must be underwound in the nucleation step, and any structural feature that makes the DNA more "unwindable" (supercoiling, high A-T content, DNA lesions) tends to enhance the rate of nucleation. The effects of such structural features are not large, however, and DNA binding to fully duplex DNA is generally measured on a time scale of hours. The only structural perturbation that substantially alters this situation is a single-strand gap 30 or more nucleotides in length. This molecular design tends to restrict RecA filament formation to regions of cellular DNA containing single-strand gaps, which in turn are regions likely to require DNA repair.

The individual strands of duplex DNA within the filament are bound asymmetrically, with one bound strand exhibiting a much greater sensitivity to nuclease treatments than the other. The better protected strand is bound at the site occupied by single-stranded DNA when RecA binds directly to that substrate. The DNA strand binding site thus defined will be referred to here as the A site. The filament is assembled $5' \rightarrow 3'$ with respect to this strand, and thus the orientation of the strand occupying the A site determines the orientation of the entire filament.

The nucleation on double-stranded DNA requires ATP but not its hydrolysis. The assembled filament again hydrolyzes ATP, but with a monomer k_{cat} that is about 30% lower than that for filaments bound to single-stranded DNA. ATP hydrolysis does not result in a cycling between the extended and collapsed forms of the filament. Instead, the filament remains at a steady state in which the DNA within the filament is underwound by 39.6%, corresponding to 17.4 base pairs per turn.

Filament disassembly is end-dependent, with dissociation of monomers taking place on the opposite end that at which monomers are added during filament extension. Both processes therefore proceed from 5′ to 3′ with respect to the DNA strand in the A site. Disassembly is conveniently monitored when RecA is bound to linear duplex DNA at neutral pH or above. Rates of disassembly increase with pH, peaking at pHs above 8.0 at a rate of 200 monomers per minute.

3.3 DNA Pairing

The homologous alignment of two DNA molecules irrespective of sequence is a molecular problem unique to homologous genetic recombination. Possible pairing intermediates in which two duplex DNAs were interwound to form a quadruplex have been discussed for more than 20 years. Most evidence now argues strongly against a four-stranded DNA pairing intermediate in RecA-mediated DNA strand exchange. Even in the four-strand exchange reactions, the initial pairing must occur with three strands within the single-strand gap of the gapped duplex.

Much evidence suggests that three-stranded DNA pairing intermediate is formed in the course of these reactions. Some evidence for a stable three-stranded structure that remains intact after RecA removal has been reported. This putative triplex DNA should have a structure quite different from known stable DNA triplexes. It is formed in a sequence-independent manner, and like strands must be arranged in parallel to meet the minimal requirements of a viable recombination intermediate. Several lines of evidence have suggested a triplex structure such as that shown in Figure 5A.

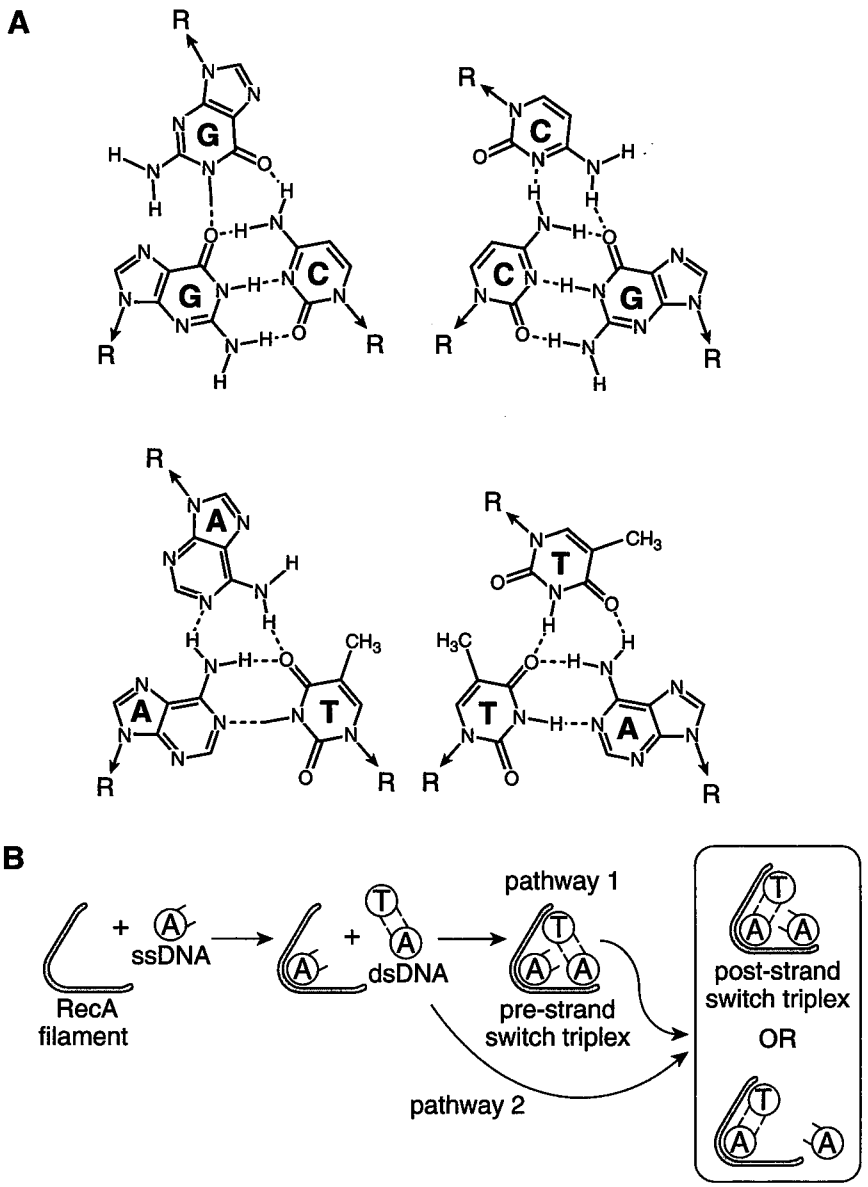


Figure 5. (A) Base pairing patterns proposed for triplex DNA intermediates in DNA strand exchange mediated by RecA protein. (B) Potential pathways for the exchange of DNA strands within a RecA filament.

There are actually two potential DNA triplex structures that could occur during a DNA strand exchange reaction (Figure 5B). Initial alignment of the two DNAs might involve formation of a pre-strand-switch triplex, in which the bound (+) single strand (DNA1) is arrayed in the major groove of the incoming duplex (DNA2) where both the + and − strands remain paired in a Watson–Crick fashion. A strand switch could then occur, pairing the − strand of DNA2 with DNA1, and leaving the displaced + strand of DNA2 arrayed in the major groove of the new hybrid duplex DNA. This would be a post-strand-switch triplex. There is no evidence for a pre-strand-switch triplex in RecA-mediated reactions. When three strands of DNA are bound within the filament, they are arranged as a post-strand-switch triplex or as hybrid DNA with a displaced plus strand. This suggests that a pre-strand-switch triplex is short-lived, or that alignment of the homologous duplex DNA is concurrent with a strand switch. A RecA protein filament tends to bind and stabilize the products of a DNA strand exchange reaction.

3.4 ATP Hydrolysis

ATP is hydrolyzed uniformly throughout the RecA filament. The K_m is on the order of 100 μM. As already noted, the turnover rate for individual monomers approaches 30 min^{-1} for filaments bound to single strands and 20–22 min^{-1} for filaments bound to duplex DNA. ATP is hydrolyzed at similar rates in a DNA-independent fashion in the presence of very high concentrations (1.5–2.0 M) of salt.

The function of ATP hydrolysis in RecA-mediated reactions remains controversial, largely because of the apparent inefficiency of the process. The strand exchange reactions depicted in Figure 4 are isoenergetic (the products and substrates contain an equivalent number of base pairs), and branch migration is a spontaneous reaction in vitro. Furthermore, a DNA strand exchange can occur through phase 3 (with the formation of 1–2 kb of hybrid DNA) in the presence of ATP analogues that are not hydrolyzed by RecA. These results show that ATP hydrolysis is not required for DNA strand exchange between homologous DNA substrates.

ATP hydrolysis *is* required for dissociation of RecA monomers from filament ends, although this activity generally accounts for only a minute fraction of ATP hydrolytic events. ATP hydrolysis is required for the extension of hybrid DNA in phase 4 of the strand exchange reaction. This is shown first by the properties of the hybrid DNA extension reaction. ATP hydrolysis renders the hybrid extension phase unidirectional, and it is required for the bypass of heterologous inserts and other structural barriers to strand exchange; both of these demonstrated functions are particularly relevant to DNA repair. Finally, ATP hydrolysis is required for strand exchange reactions involving four strands. More direct evidence for a coupling between ATP hydrolysis and the extension of hybrid DNA in phase 4 of DNA strand exchange is found in the observation that the rates of the processes exhibit identical dependencies on temperature over the range of 25–45°C.

3.5 Models

Mechanistic ideas for DNA strand exchange began with a proposal of P. Howard-Flanders in 1984 that DNA pairing and strand switching occur within the major helical groove of the RecA filament. Newer models built on this idea have provided mechanisms by which ATP hydrolysis might be coupled to DNA strand exchange.

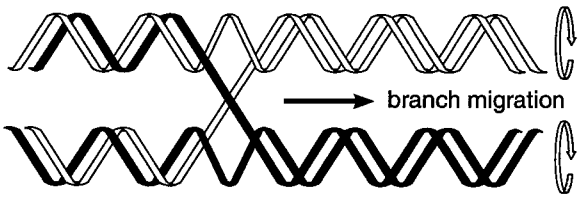


Figure 6. Movement of a branch connecting two homologous and helical DNA molecules requires rotation of the DNAs as shown.

One suggestion is that discontinuities in the RecA filament limit the progress of ATP-independent DNA strand exchange in phase 3. ATP hydrolysis would then be required simply to redistribute the RecA protein. Continued extension of the hybrid DNA in phase 4 would depend on available contiguous RecA filament and remain largely ATP-independent.

A second model is derived from the requirement that the two DNA substrates rotate if DNA strand exchange is to take place (Figure 6). The most straightforward way to facilitate strand exchange is to couple ATP hydrolysis directly to this rotation (Figure 7). If one of the two DNA substrates binds to the outside of the filament, even over a short region, the rotation can be brought about by passing this external segment between monomers in a ratchetlike action coupled to ATP hydrolysis. In Figure 7A, the homologous duplex DNA is first aligned within the helical filament groove to form a region of paired DNA. Any DNA still outside the filament binds to one of six "motor" sites lining the exterior of the filament longitudinally. Rotation of the DNA is effected by passing the exterior DNA from one site to another around the outside of the filament. There are six RecA monomers per turn in the filament. Each 360° rotation would occur in six steps, with one-sixth of the RecA monomers in the filament participating in each step (filament-end view, Figure 7B). Since DNA within a RecA filament is underwound to 18 bp per turn, each complete rotation would move the DNA branch by 18 bp. A unidirectional rotation would necessarily result in unidirectional strand exchange. In this model, ATP-independent strand exchange is limited not by discontinuities in the filament, but by limits to the contiguous length of DNA that is paired within the filament in phase 3, with the extension phase coupled to the ATP-dependent rotation.

The function of ATP hydrolysis in DNA strand exchange mediated by RecA protein remains a controversial and unresolved issue. These proposals and others that may appear provide the testable predictions and experimental focus needed for progress on this question.

The importance of the mediation of the DNA strand exchange reaction by RecA protein as a paradigm has been enhanced by the recent discovery that the Rad51 protein promotes a very similar DNA strand exchange reaction in vitro. More and more frequently, prokaryotic enzyme systems involved in DNA metabolism are proving to be good models for the analogous processes in eukaryotes.

4 RecA PROTEIN AS A COPROTEASE

Extensive DNA damage in bacteria triggers a global cellular response mediated by the SOS regulatory system. The key components of the regulatory system are the LexA repressor, which regulates the expression of the genes that are induced in SOS, and the

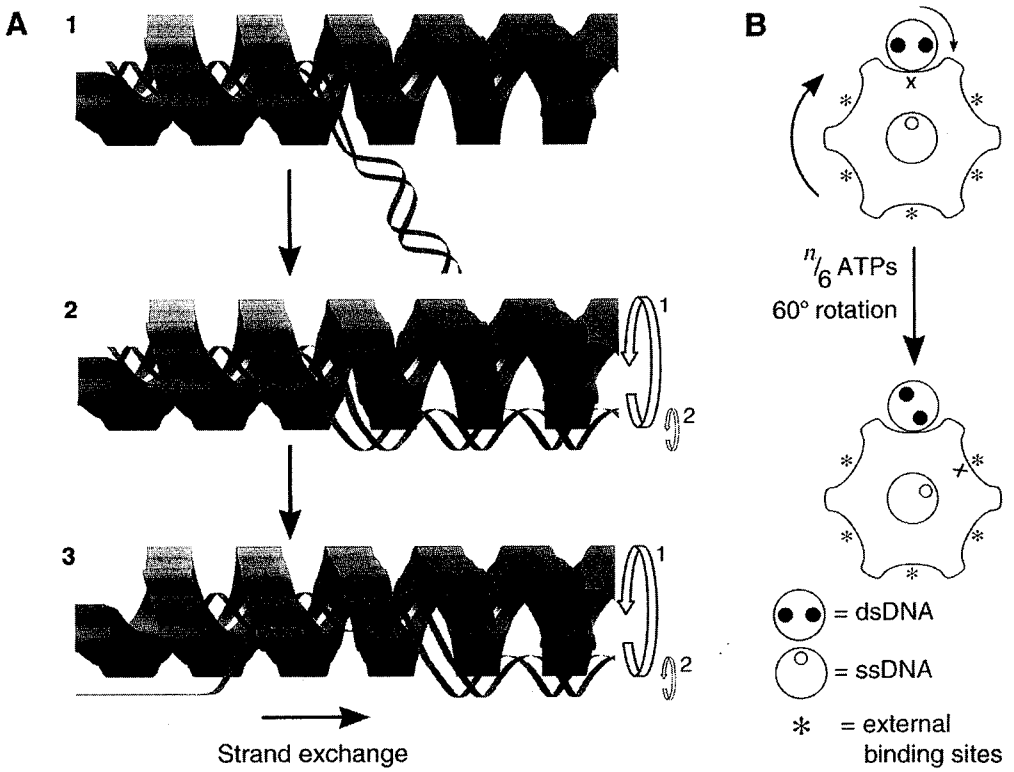


Figure 7. (A) A model for RecA-facilitated and ATP-dependent DNA strand exchange. Rotation of one of the DNA molecules around the outside of the filament, using a ratchetlike action of RecA monomers coupled to ATP hydrolysis, could produce a unidirectional DNA strand exchange. (B) An end view showing how the rotation could occur in six steps (the RecA filament contains six RecA monomers per turn). This model was described in detail by Cox in 1992.

RecA protein. Induction of the SOS system requires the inactivation of the lexA repressor, which comes about via cleavage at a specific Ala-Gly bond near the center of the repressor. The cleavage is catalyzed by LexA itself, and occurs spontaneously at high pH. At neutral pH the cleavage is facilitated by RecA protein. The signal for induction of the SOS system is the appearance single-stranded DNA gaps as a result of DNA damage. The RecA nucleoprotein filament that assembles on the DNA in these circumstances is the active form that facilitates the cleavage of LexA. Since RecA does not act as a classical protease, but instead facilitates an autocatalytic cleavage of LexA, R. Devoret introduced the term "coprotease" to describe this RecA activity.

5 OTHER RECOMBINATION PROTEINS

More than 20 different proteins play some role in homologous genetic recombination in *E. coli* and other bacteria. In addition to the products of the genes listed in Section 1.1, a variety of enzymes that act during DNA replication, including DNA ligase, the single-strand DNA-binding protein (SSB), and topoisomerases, must also function during recombination and recombinational repair. SSB plays an especially prominent role, greatly stimulating the activities of RecA protein and several of the proteins described in the subsections that follow.

There are several alternative pathways for recombination in prokaryotes. RecA protein plays a central role in most, but not all of them. The pathways are defined by their requirements for individual proteins. Studies are advancing rapidly in the characterization of many recombination proteins. Some that appear to be uniquely involved in recombinational DNA repair and recombination are summarized next.

5.1 Proteins Involved in the Initiation of Recombination

5.1.1 The RecBCD Enzyme

The *recB, C,* and *D* genes encode proteins with molecular masses of 134, 129, and 67 kDa, respectively. These proteins function as subunits of a large enzyme that possesses ATP-dependent DNA helicase and nuclease activities. The RecBCD enzyme functions primarily during the initiation of recombinational DNA repair at double-strand breaks and recombination during conjugation and transduction. Mutations in the *recB* or *recC* genes reduce levels of conjugational recombination or transduction by two to three orders of magnitude. RecBCD also contributes to the degradation of foreign DNA entering the cell. Recombination processes dependent on RecBCD function are said to be part of the RecBCD pathway of recombination.

The ends of double-stranded DNA provide loading sites for the RecBCD enzyme. The enzyme can processively unwind an average of 30 kb of DNA at rates exceeding 1000 base pairs per second.

About 1–3 molecules of ATP are hydrolyzed per unwound base pair. Under some conditions, the enzyme also degrades the single strands that it generates, probably by endonucleolytic cleavage behind the point of double-stranded DNA unwinding. The result is the generation of single-stranded DNA fragments with lengths that vary with reaction conditions.

The activity of the RecBCD enzyme is modulated by interaction with an eight-nucleotide DNA sequence called χ (5′ GCTGGTGG). When RecBCD approaches the χ sequence from the 3′ side, it cuts the χ-containing strand four to six nucleotides away from it on the 3′ side. Interaction with χ also alters the RecBCD enzyme, attenuating its exonuclease activity at least 500-fold. The enzyme continues to move down the DNA, unwinding but not degrading it.

The overall effect of RecBCD action is to generate single-stranded DNA, which can then be bound by RecA protein and used in DNA strand exchange. A possible involvement of RecBCD enzyme at other stages of recombination remains controversial.

5.1.2 The RecE and RecT Proteins

When cells are deficient in RecBCD activity, nearly normal levels of recombination can be restored by introducing a secondary mutation called *sbcA* (suppressor of recBC). The *sbcA* mutation activates two open reading frames, the *recE* and *recT* genes, in a cryptic prophage present in most *E. coli* strains. The RecE protein is a 140 kDa polypeptide that functions as a trimer or tetramer. It has an exonuclease activity (and is designated exonuclease VIII), degrading the 5′ terminal strand in double-stranded DNA. The RecT protein (33 kDa) binds to single-stranded DNA and can promote ATP-independent renaturation with a complementary DNA strand or DNA strand exchange. Recombination dependent on these proteins defines the RecE pathway of recombination. In some instances, such as plasmid recombination, this pathway operates independently of RecA protein.

5.1.3 Exonuclease I

There are additional suppressors of *recBC* mutations, including *sbcB, sbcC,* and *sbcD* mutations. The function of the products of the latter two genes is not known. The *sbcB* gene encodes exonuclease I (72 kDa), which specifically degrades single-stranded DNA from the 3′ end. The *sbcB* mutation inactivates the exonuclease activity, and it is thought that this might stabilize single-stranded DNA with 3′ ends, which in turn provide good substrates with which to initiate recombination.

5.1.4 The RecF, O, and R Proteins

In cells with *recBC* and *sbcBC* mutations, most recombination occurs via a third route called the RecF recombination pathway. Three proteins usually identified with this pathway are RecF (40 kDa), RecO (26 kDa), and RecR (22 kDa). These proteins are believed to function early in recombination. Mutations in the *recF, O,* or *R* genes render the cells sensitive to DNA-damaging agents and defective in plasmid recombination. Defects in other types of recombination are not evident except in the $recBC^{-}sbcBC^{-}$ genetic backgrounds. There are several lines of genetic evidence that the three proteins act together in the cell. RecF protein binds to ATP and to DNA, but no activity has been described for it other than a general inhibition of reactions promoted by RecA. The RecO and RecR proteins act together in vitro to facilitate the binding of RecA proteins to single-stranded DNA coated with SSB. Additional activities may come to light.

5.1.5 The RecJ Protein

The 63 kDa RecJ protein is an ATP-independent exonuclease that degrades single-stranded DNA from the 5′ end. This enzyme is required in both the RecE and RecF recombination pathways, and mutations in the *recJ* gene substantially reduce the efficiency of plasmid recombination.

5.1.6 The RecQ Protein

The *recQ* gene encodes a DNA helicase activity that is generally associated with the RecF recombination pathway. In recBCD-deficient cells, the 67 kDa *RecQ* protein may replace the RecBCD helicase activity. The human gene responsible for Bloom's Syndrome has been shown to be a *recQ* homologue.

5.2 Proteins That Act in the Later Stages of Recombination

5.2.1 The RuvA and B Proteins

Mutations in the *ruvA* and *ruvB* genes increase a cell's sensitivity to UV radiation by two to four orders of magnitude and exhibit a sensitivity to many other DNA-damaging agents. The isolated proteins (22 and 37 kDa, respectively) act together to process branched DNA molecules that represent recombination intermediates generated largely by RecA action. RuvA protein binds to branched DNA molecules and facilitates the binding of RuvB protein. The RuvB protein hydrolyzes ATP, has a DNA helicase activity, and promotes the rapid branch migration of Holliday junctions. The proteins probably play a major role in the latter stages of recombination. The proteins also exhibit a capacity to resolve or reverse the formation of recombination intermediates when DNA strand exchange has stalled at a DNA structural barrier. In the cell, these proteins may do more than contribute to productive recombination events. In cases of recombination initiated in a manner that might be deleterious to the cell (e.g., recombination between repeated sequence elements on the chromosome, which might result in a substantial genomic deletion), these proteins might play a protective role and reverse the reactions.

5.2.2 The RecG Protein

Mutations in the *recG* gene exhibit only modest effects on recombination, and these effects are enhanced in a $recBC^{-}sbcBC^{-}$ genetic background. The effects of a *recG* deficiency are most evident, however, when it is combined with *ruvA* or *B* mutations, producing drops in the efficiencies of transduction and conjugational recombination by several orders of magnitude. The RecG protein (76 kDa) appears to provide an alternative pathway for the processing of branched recombination intermediates. In vitro, the protein exhibits a potent DNA-dependent ATPase activity. Like RuvA and B, the RecG protein facilitates branch migration of Holliday junctions.

5.2.3 The RuvC Protein

The RuvC protein is an endonuclease that specifically recognizes and cleaves Holliday intermediates. The DNA cuts are symmetric,

arranged so that two viable recombination products are produced. The careful characterization of this 19 kDa enzyme in vitro has provided much evidence that it is one activity required to resolve Holliday intermediates in the final stages of recombination.

5.2.4 Other Proteins

The activities of many of the other proteins involved in recombination are unknown or incompletely characterized. Reconstitution of a complete recombination system in vitro is one goal of continuing research in this field.

See also BACTERIAL GROWTH AND DIVISION; DNA DAMAGE AND REPAIR; *E. COLI* GENOME; RECOMBINATION; MOLECULAR BIOLOGY OF.

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RECEPTOR BIOCHEMISTRY

Tatsuya Haga

1 Classification of Receptors

2 Ion Channel–Coupled Receptors (ICR)
- 2.1 Nicotinic Acetylcholine Receptors
- 2.2 Glutamate Receptors
- 2.3 GABA Receptors

3 G-Protein–Coupled Receptors (GPR)
- 3.1 Structure, Function, and Regulation of GPRs
- 3.2 G-Protein Species
- 3.3 Classification of GPRs and Specificity of GPR–G-Protein Interaction
- 3.4 Neurotransmitter Receptors: Roles of ICRs and GPRs

4 Protein Kinase–Coupled Receptors (PKR)
- 4.1 Growth Factor Receptors with Tyrosine Kinase Activity
- 4.2 Cytokine Receptors Linked with Tyrosine Kinases
- 4.3 TGF-β Receptors with Serine/Threonine Kinase Activity
- 4.4 Intracellular Signaling: Common Pathways Triggered by GPRs and PKRs

5 Other Receptors

Key Words

Agonists Compounds that bind to and activate receptors and include endogenous ligands such as hormones and neurotransmitters, chemically synthesized compounds, and natural products like alkaloids.

Antagonists Compounds that bind to but do not activate receptors, hence inhibit the action of agonists competitively.

Cytokines and Growth Factors Proteins that influence the proliferation, differentiation, maturation, and survival of cells. Cytokines include interleukins (IL), which are produced by leukocytes and control leukocyte function, and interferons (IFN), which are produced in response to virus infection. Growth factors are produced constitutively and act on nonhematopoietic cells, whereas cytokines are produced transiently and are mostly involved in hematopoiesis and immune or inflammatory responses. In a broader sense, cytokines include growth factors.

GTP-Binding Regulatory Proteins (G Proteins) Proteins that are activated by hormone- or neurotransmitter-bound receptors and activates effectors such as adenylate cyclase, phospholipase C, and ion channels. G proteins are composed of an α subunit with GTP binding activity and βγ subunits.

Hormones Chemical substances that are released from one cell and modulate the function of other cells which are usually located in remote organs.

MAP Kinase (MAPK) Mitogen-activated protein kinase, a serine/threonine kinase in the signal transduction pathway that is triggered by activation of growth factor receptors. Extracellular-signal-regulated kinase is another name and is abbreviated ERK. MAP kinase kinase [MAPKK or MEK (MAP/ERK kinase)] phosphorylates both tyrosine and threonine residues in MAP kinase and activates MAP kinase. MAPKK kinase (MAP