Table 6 Use and Sensitivity of Autoradiography and Fluorography

Isotope	Method	Example Applications	Preflashed Film	Intensifying Screen	Temperature of Exposure	Approximate Detection Limit After 24 h Exposure (dpm/cm^2)
^3H	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, or ^{33}P	Direct	DNA sequencing	No	No	Room temp	6×10^3
	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

of light as they return to a ground state. The energy of radiation from ^{32}P is high enough to cause this Cerenkov effect. Consequently water can be used in place of scintillation fluid for this radioisotope. The counting efficiency is relatively low (about 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 ^3H. The big advantages of Cerenkov counting are that aqueous samples can be counted and recovered for experimental use, and there is no accumulation of organic radioactive waste.

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) and detection of particular clones or plaques by hybridization screening. The basic principle is that light, a β-particle, X-rays, or γ-rays can produce silver atoms in crystals of the silver halide present in photographic emulsions. Fluorography is an adaptation of the technique in which radioactivity is detected by light emanating from a scintillator (the "fluor"), which is either incorporated into the sample (e.g., PPO in gels emitting radiation of low energy) or provided as an intensifying screen in autoradiography cassettes (often referred to as indirect autoradiography). Fluorography increases sensitivity but reduces resolution.

A summary of some of the key factors of autoradiography and fluorography is provided in Table 6.

6.5 Personal Dosimeters

Individuals who are at risk from occupational exposure to potentially hazardous levels of ionizing radiation may be issued film badges or thermoluminescent detectors (TLDs). The latter contain a phosphor such as LiF, which becomes and remains excited once exposed to radiation. Heat treatment results in light emission, the intensity of which is related to the dose received. Small TLDs can be worn on the fingers, under gloves, for hand dosimetry.

Personal dosimeters are not appropriate for workers using low energy emitters such as ^{14}C, ^{35}S, or ^3H, 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 dosimetry calculations and estimates of exposure levels to individuals.

See also Labeling, Biophysical.

Bibliography

Ballance, P. E., Day, R. L., and Morgan, J. (1992) *Phosphorus-32: Practical Radiation Protection.* H and H Scientific Consultants, Leeds.

Mundy, C. R., Cunningham, M. W., and Read, C. A. (1991) Nucleic acid labelling and detection. In *Essential Molecular Biology A Practical Approach,* T. A. Brown, Ed. Oxford University Press, Oxford and New York.

International Commission on Radiological Protection. (1991) ICRP60: Recommendations of the International Commission on Radiological Protection. *Annals of the ICRP,* Vol. 21, Nos. 1–3. Pergamon Press, Oxford.

———. (1991) ICRP 61: Annual limits on intake of radionuclides by workers based on 1990 recommendations. *Annals of the ICRP,* Vol. 21, No. 4. Pergamon Press, Oxford.

Slater, R. J., Ed. (1990) *Radioisotopes in Biology: A Practical Approach.* Oxford University Press, Oxford and New York.

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

RecA Protein, Structure and Function of

Michael M. Cox

Key Words

Genetic Recombination Rearrangement of genetic information within or between nucleic acids.

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

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

Holliday Intermediate 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.

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 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 recombination.

1 OVERVIEW

The product of the *rec*A gene is a multifunctional protein that occupies an important crossroad in bacterial DNA metabolism, with important roles in DNA repair, recombination, and replication. Biochemical interest is focused on the mechanism(s) by which RecA protein mediates its varied functions in DNA metabolism.

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 to map genes. The molecular process underlying these genetic exchanges is known as homologous genetic recombination.

A generic model illustrating the kinds of molecular processes 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. The 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 repaired.

The first genes with products involved in recombination were identified in *E. coli* in 1965 by A. J. Clark. Recombination genes now include *rec*A, *rec*B, *rec*C, *rec*D, *rec*F, *rec*G, *rec*J, *rec*N, *rec*O, *rec*Q, *rec*R, *ruv*A, *ruv*B, *ruv*C, and *ssb*. Mutations in the *rec*A gene exhibit the most complex phenotypes. The product of this gene is a multifunctional protein called the RecA protein.

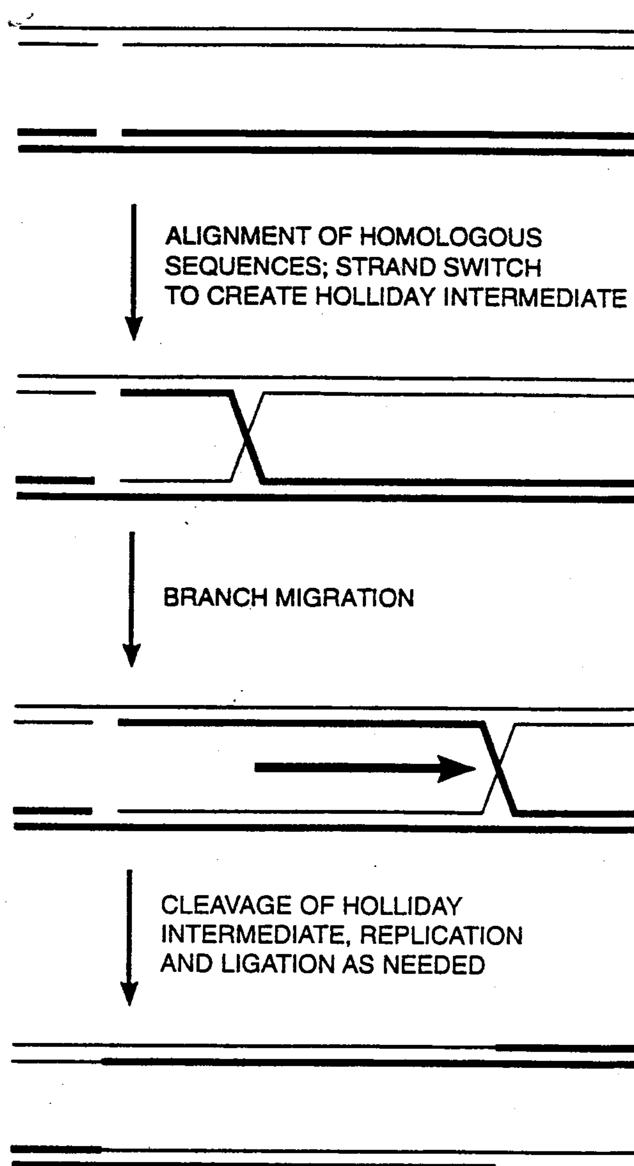

Figure 1. One model for homologous genetic recombination between two homologous chromosomes, represented by thick and thin lines. Regions of the DNA intermediates and products in which thick and thin lines are paired are called heteroduplex DNA. There are many variants of this model, and the depiction is designed only to show the kinds of processes that must occur to bring about recombination.

1.2 Biological Function of RecA

The immediate selective value of RecA protein to the bacterial cell probably lies in its recombinational repair function. In each cell generation, a bacterial cell growing in an aerobic environment is subjected to thousands of DNA lesions, 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 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. The molecular

form of RecA is adapted to render recombinational DNA repair maximally efficient.

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.

RecA protein also has a third, and quite different function. It facilitates the autocatalytic cleavage of the *lex*A repressor to induce a response to abnormally extensive DNA damage called the SOS response.

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 *rec*A gene has been sequenced in more than 40 bacterial species. The primary structure is illustrated in Figure 2, with 71 invariant amino acid residues highlighted. The close relationship between bacterial RecA proteins is reinforced by sequence similarities at many other positions. A wide range of RecA mutants are available, and these have facilitated an understanding of structure–function relationships in this polypeptide.

The wide distribution of bacterial *rec*A 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. Eukaryotic genes with significant sequence homology to *rec*A have been found in yeast and plants.

2.2 THREE-DIMENSIONAL STRUCTURE

The structure of RecA protein has been determined at 2.3 A resolution by R. Story and T. Steitz. There is a major central domain flanked by two smaller subdomains at the N- and C-termini. The central domain contains a single binding site for ATP or ADP. 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 14). 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.

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; it is easy to distinguish products from substrates with a wide range of assays. 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 (cf. Figures 1 and 4). A Holliday intermediate is formed transiently in the four-strand reaction.

3.1 FUNDAMENTALS

The active species promoting DNA strand exchange is a RecA nucleoprotein filament that completely coats the single-stranded or gapped DNA substrate as the first step in the reaction. The bound single strand is paired to one end of the linear duplex DNA to create the branched molecules shown in Figure 4. The heteroduplex DNA thus created is then extended in a facilitated branch migration reaction that proceeds until products are formed. The branch migration is unidirectional (5′ to 3′ with respect to the single strand initially bound), and proceeds at a rate of three to six base pairs per second. RecA-mediated DNA strand exchange proceeds readily past mismatches, lesions, and even heterologous inserts (up to a few hundred base pairs long) 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 a hydrolyzed during strand exchange. The apparent efficiency of the reaction is low, with about 100 ATP molecules hydrolyzed per base pair of heteroduplex formed under typical reaction conditions. The manner in which the two DNA molecules are aligned to begin strand

AIDENKQKA**L**	AAALGQIEKQ	F**GKG**SI**M**RLG	EDRSMDVETI	40
STGSLS**L**DI**A**	L**G**AGGLPMGR	IV**E**IY**GPES**S	**GKTT**LTLQVI	80
AAAQREGKTC	AFIDAEH**A**L**D**	PI**Y**ARK**LG**VD	IDN**L**LCSQ**P**D	120
TGEQALE**I**CD	ALARSGAV**D**V	IVV**DSVAAL**T	**P**KA**E**IE**G**EIG	160
DSHM**G**LA**AR**M	M**SQA**M**R**KLAG	NLKQSNTLLI	**F**I**NQ**I**R**M**K**IG	200
VMF**G**N**PE**T**T**T	**GG**N**ALKF**YA**S**	**VR**LDI**R**RIGA	V**K**EGENVV**G**S	240
ETRV**KV**V**K**NK	IAA**P**FKQAEF	Q**I**LYGE**G**INF	YGELVDLGVK	280
EKLIEKA**G**A**W**	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 40 sequenced bacterial *rec*A genes.

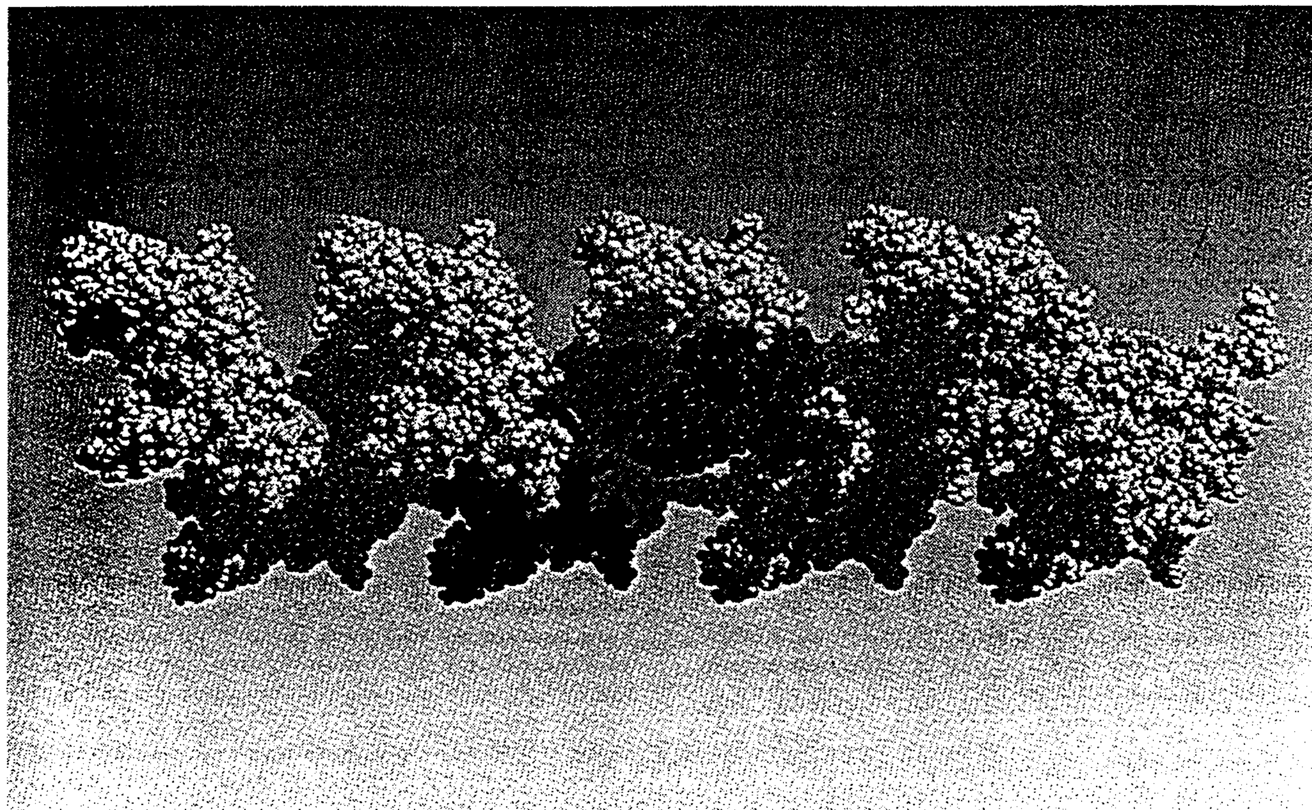

Figure 3. Representation of a segment of a helical filament formed by 24 monomers of RecA protein. Six monomers (one helical turn) are colored. [Illustration based on the structure determined by Story et al. (1992) and developed from the coordinates deposited in the Brookhaven Protein Data Bank, using the MidasPlus software obtained from the Computer Graphics Laboratory at the University of California, San Francisco.] (See color plate 14.)

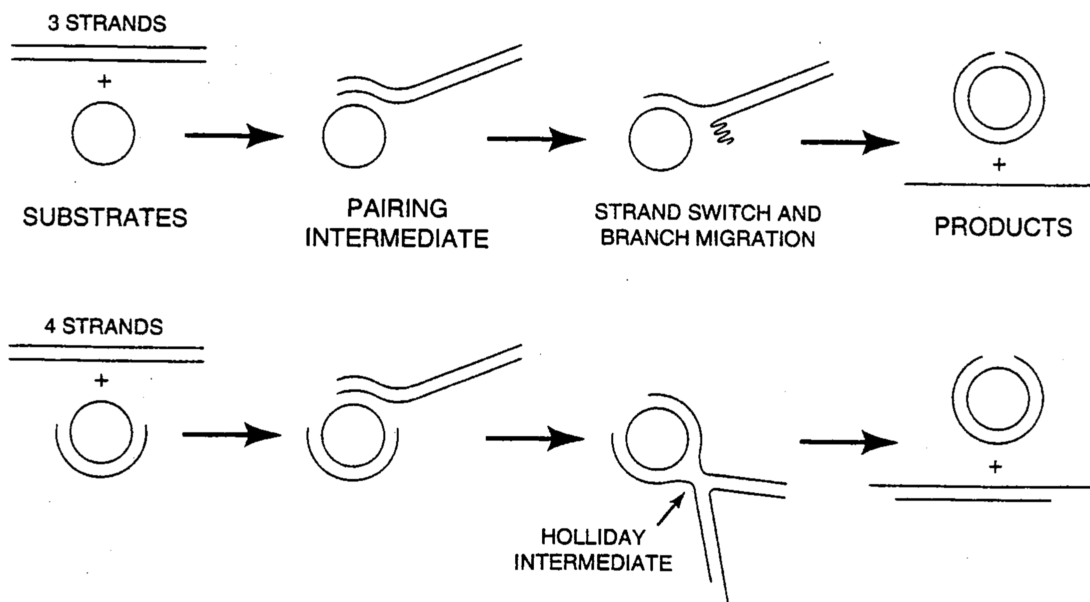

Figure 4. DNA strand exchange reactions promoted by RecA protein in vitro; thin lines represent individual DNA strands.

exchange, and the molecular role of ATP hydrolysis, remain as major unresolved mechanistic questions.

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 A 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 much more rapidly to single strands than to duplex DNA. Following nucleation, the filament is extended in the 5′ to 3′ direction along the DNA. If the substrate is a duplex with a single-strand gap, the nucleation occurs in the gap and the filament is extended rapidly to encompass the adjacent duplex. Nucleation on duplex DNA is very slow, although filament extension is rapid once nucleation has occurred. 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.

Where it occurs, dissociation of RecA monomers from a filament takes place on the end opposite to that at which monomers are added during filament extension.

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 a three-stranded DNA pairing intermediate is formed in the course of these reactions. Some evidence has been reported for a stable three-stranded structure that remains intact after RecA removal. 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.

3.4 ATP Hydrolysis

ATP is hydrolyzed uniformly throughout the RecA filament. The Michaelis constant K_m is on the order of 100 μM. The turnover rate for individual monomers approaches 30 per minute for filaments bound to single strands and 20–22 per minute for filaments bound to duplex DNA. ATP is hydrolyzed at similar rates in a DNA-dependent 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 its apparent inefficiency. 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, substantial DNA strand exchange can occur 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 category accounts for only a small fraction of ATP hydrolytic events. ATP hydrolysis renders the strand exchange reaction unidirectional and is required for the bypass of heterologous inserts and other structural barriers to strand exchange; both these demonstrated functions are particularly relevant to DNA repair. Finally, ATP hydrolysis is required for strand exchange reactions involving four strands. A molecular explanation for these requirements has not been established.

3.5 Models

Mechanistic ideas for DNA strand exchange begin with a proposal of P. Howard-Flanders that DNA pairing and strand switching occur within the major helical groove of the RecA filament. Much evidence indicates that DNA pairing and probably the strand switch to form heteroduplex DNA occur within the groove in a process that requires ATP but not its hydrolysis. ATP hydrolysis is required at a later stage, for dissociation of RecA monomers following reaction and/or for some molecular process that renders the reaction unidirectional and forces strand exchange past structural barriers such as heterologous inserts. A model has been proposed that couples ATP hydrolysis to a coordinated rotation of the two DNA substrates to effect a unidirectional strand exchange that could bypass such barriers.

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 *lex*A repressor, which regulates the expression of the genes that are induced in SOS, and the RecA protein. Induction of the SOS system requires the inactivation of the *lex*A repressor, which comes about via cleavage at a specific Ala-Gly bond near the center of the repressor. The cleavage is catalyzed by *lex*A itself, and it 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 of 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 *lex*A. Since RecA does not act as a classical protease, but instead facilitates an autocatalytic cleavage of *lex*A, Raymond 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, including the products of the genes listed in Section 1.1 and a variety of enzymes that also act during DNA replication (such as DNA ligase and topoisomerases). Only a few of the recombination-specific proteins have been studied in any detail. The *rec*B,C, and D genes encode a DNA helicase/nuclease (the recBCD enzyme) that functions primarily during the initiation of recombinational DNA repair at double-strand breaks and recombination during conjugation and transduction. The RecJ protein is an exonuclease that degrades

single-stranded DNA 5′ to 3′. The recQ protein is a DNA helicase. The RuvC protein is an endonuclease that specifically recognizes and resolves Holliday intermediates. 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.

Bibliography

Cox, M. M. (1993) Relating biochemistry to biology: How the recombinational repair function of the recA system is manifested in its molecular properties. *BioEssays,* 15:617–623.

Cox, M. M. (1994) Why does RecA protein hydrolyze ATP? *Trends Biochem. Sci.* 19:217–222.

Kowalczykowski, S. C., Dixon, D. A., Eggleston, A. K., Lauders, S. D., and Rehrauer, W. M. (1994) Biochemistry of homologous recombination in *Escherichia coli. Microbiol. Rev.* 58:401–465.

Kucherlapati, R., and Smith, G. R., Eds. (1988) *Genetic Recombination.* American Society for Microbiology, Washington, DC.

Little, J. W. (1991) Mechanism of specific LexA cleavage—Autodigestion and the role of RecA coprotease. *Biochimie,* 73:411–422.

Radding, C. M. (1991) Helical interactions in homologous pairing and strand exchange driven by RecA protein *J. Biol. Chem.* 266:5355–5358.

Roca, A. I., and Cox, M. M. (1990) The RecA protein: Structure and Function. *CRC Crit. Rev. Biochem. Mol. Biol.* 25:415–456.

Smith, G. R. (1989) Homologous recombination in *E. coli:* Multiple pathways for multiple reasons. *Cell,* 58:807–809.

Smith, K. C., and Wang, T. C. (1989) recA-dependent DNA repair processes. *BioEssays,* 10:12–6.

Story, R. M., Weber, I. T., and Steitz, T. A. (1992) The structure of the *E. coli* RecA protein monomer and polymer. *Nature,* 355:318–325.

West, S. C. (1992) Enzymes and molecular mechanisms of recombination. *Annu. Rev. Biochem.* 61:603–640.

Receptor Biochemistry

Tatsuya Haga

Key Words

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

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

GTP-Binding Regulatory Proteins (G Proteins) Proteins that are activated by agonist-bound receptors and activate effectors such as adenylate cyclase, phospholipase C, and ion channels.

Second Messengers Compounds that are formed in the cells in response to stimulation by agonists on the surface of cells (e.g., cyclic AMP, cyclic GMP, diacylglycerol, inositol triphosphate).

Communications between the cells are performed by cell–cell contact or by chemical substances like hormones, autocoids, or neurotransmitters. These chemical substances are secreted by a cell and recognized by receptors in target cells. The binding of the ligands to their receptors initiates a series of chemical reactions. Some hormones such as steroids and thyroxine pass through cell membranes and interact with their receptors in the cells. Most other hormones and all known neurotransmitters cannot pass through cell membranes and bind to their receptors, which are transmembrane glycoproteins, on the surface of cells. Membrane-bound receptors are classified into three major groups: ion channel receptors, G-protein-linked receptors, and other receptors for growth factors or other proteins. This entry describes the molecular properties of membrane-bound receptors.

1 CLASSIFICATION OF RECEPTORS

Receptors have been named and classified by their endogenous ligands and subclassified by specific agonists and antagonists since the introduction of the concept of receptor in the early 1900s by Langley. The molecular entities of receptors were identified in the last decade, enabling us to classify receptors on the basis of structure and function. For example, receptors for acetylcholine have been classified as nicotinic acetylcholine receptors and muscarinic acetylcholine receptors on the basis of their interactions with specific agonists, nicotine and muscarine, respectively. Purification of these receptors and their functional reconstitution into artificial membranes showed that the nicotinic receptor is an acetylcholine-gated ion channel, while the muscarinic receptor is an activator of G proteins, there is no structural or functional similarity between the two. Cloning of complementary DNAs encoding receptor proteins and deduction of their amino acid sequences revealed that a number of receptors have structural characteristics similar to those of the nicotinic or muscarinic receptor and that they constitute two superfamilies of ion channel receptors and G-protein-linked receptors. The structural characteristics common to members of the two superfamilies are numbers of hydrophobic regions that are supposed to be transmembrane segments: subunits of ion channel receptors and G-protein-linked receptors have four and seven such regions, respectively. On the other hand, several groups of receptors for growth factors or other proteins are characterized by a single transmembrane segment.

Cloning of cDNAs encoding receptor proteins has revealed that the number of subtypes for any given receptor is greater than that of subtypes identified by specific ligands. The extreme case is the odorant receptors: more than 100 receptors are reported to exist, and 18 cDNAs encoding odorant receptors have been cloned, but their ligands remain to be identified. One of the challenging projects in the next decade will be to conjecture and synthesize specific ligands for a given receptor with a known amino acid sequence.

2 ION CHANNEL RECEPTORS

Endogenous ligands of ion channel receptors are the following neurotransmitters: acetylcholine, glutamate, γ-aminobutyric acid (GABA), glycine, serotonin, and probably ATP. Ion channel receptors are not known for catecholamines like adrenaline and dopamine, nor for peptides (Tables 1 and 2).

Ion channel receptors are oligomers composed of heterogeneous subunits, although some might be homooligomers, and they incor-