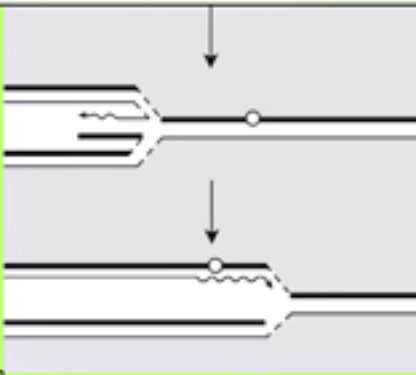
ADVANCES IN MOLECULAR AND CELLULAR MICROBIOLOGY

AMCM

The Dynamic Bacterial Genome



Edited by
Peter Mullany

CAMBRIDGE

CAMBRIDGE www.cambridge.org/9780521821575

This page intentionally left blank

The Dynamic Bacterial Genome

This book provides an in-depth analysis of the mechanisms and biological consequences of genome rearrangements in bacteria. Genome rearrangements take place as a result of the actions of discrete genetic elements such as conjugative transposons, plasmids, phage, and nonconjugative transposons. Bacteria also contain systems to mediate genetic rearrangements such as the general recombination pathway and specialized endogenous recombination mechanisms. The biological effect of these rearrangements is far-reaching and impacts on bacterial virulence, antibiotic resistance, and the ability of the bacteria to avoid the attentions of the host immune system (e.g., antigenic variation). These rearrangements also provide the raw material on which natural selection can act.

Each chapter examines the mechanisms involved in genome rearrangements and the direct biological consequences of these events. Because genome rearrangements are so important in evolution, at least one of the chapters views the phenomenon from an evolutionary angle. This book provides the reader with a holistic view of genome rearrangements (i.e., studies on both the biological consequences of genome rearrangement and the mechanisms underlying these processes are presented).

The book is written by leading research workers in the field and is aimed at final-year undergraduates, postgraduate and postdoctoral workers, and established biologists.

Peter Mullany is a Reader in Molecular Microbiology at University College London. His main research interests are the molecular biology and molecular ecology of conjugative transposons. He has been at the forefront of this work for the past 10 years and has pioneered the genetic analysis of the important human pathogen *Clostridium difficile*.

Over the past decade, the rapid development of an array of techniques in the fields of cellular and molecular biology have transformed whole areas of research across the biological sciences. Microbiology has perhaps been influenced most of all. Our understanding of microbial diversity and evolutionary biology, and of how pathogenic bacteria and viruses interact with their animal and plant hosts at the molecular level, for example, have been revolutionized. Perhaps the most exciting recent advance in microbiology has been the development of the interface discipline of cellular microbiology, a fusion of classic microbiology, microbial molecular biology, and eukaryotic cellular and molecular biology. Cellular microbiology is revealing how pathogenic bacteria interact with host cells in what is turning out to be a complex evolutionary battle of competing gene products. Molecular and cellular biology are no longer discrete subject areas but vital tools and an integrated part of current microbiological research. As part of this revolution in molecular biology, the genomes of a growing number of pathogenic and model bacteria have been fully sequenced, with immense implications for our future understanding of microorganisms at the molecular level.

Advances in Molecular and Cellular Microbiology is a series edited by researchers active in these exciting and rapidly expanding fields. Each volume focuses on a particular aspect of cellular or molecular microbiology and provides an overview of the area, as well as examines current research. This series will enable graduate students and researchers to keep up with the rapidly diversifying literature in current microbiological research.

ADVANCES IN MOLECULAR AND CELLULAR MICROBIOLOGY

AMCM

Published Titles

1. *Bacterial Adhesion to Host Tissues*. Edited by Michael Wilson 0521801079
2. *Bacterial Evasion of Host Immune Responses*. Edited by Brian Henderson and Petra Oyston 0521801737
3. *Dormancy and Low-Growth States in Microbial Disease*. Edited by Anthony R. M. Coates 0521809401
4. *Susceptibility to Infectious Diseases*. Edited by Richard Bellamy 0521815258
5. *Bacterial Invasion of Host Cells*. Edited by Richard J. Lamont 0521809541
6. *Mammalian Host Defense Peptides*. Edited by Deirdre Devine and Robert Hancock 0521822203
7. *Bacterial Protein Toxins*. Edited by Alistair J. Lax 052182091X

Forthcoming Titles in the Series

The Influence of Bacterial Communities on Host Biology. Edited by Margaret McFall-Ngai, Brian Henderson, and Edward Ruby 0521834651

The Yeast Cell Cycle. Edited by Jeremy Hyams 0521835569

Salmonella Infections. Edited by Pietro Mastroeni and Duncan Maskell 0521835046

Phagocytosis of Bacteria and Bacterial Pathogenicity. Edited by Joel Ernst and Olle Stendahl 0521845696

Quorum Sensing and Bacterial Cell-to-Cell Communication. Edited by Donald R. Demuth and Richard J. Lamont 0521846382

Advances in Molecular and Cellular Microbiology, Volume 8

The Dynamic Bacterial Genome

EDITED BY
PETER MULLANY
University College London

CAMBRIDGE
UNIVERSITY PRESS

CAMBRIDGE UNIVERSITY PRESS
Cambridge, New York, Melbourne, Madrid, Cape Town, Singapore, São Paulo

Cambridge University Press
The Edinburgh Building, Cambridge CB2 8RU, UK
Published in the United States of America by Cambridge University Press, New York
www.cambridge.org
Information on this title: www.cambridge.org/9780521821575

First published in print format 2005

ISBN-13 978-0-511-34329-2 eBook (Adobe Reader)
ISBN-10 0-511-34329-9 eBook (Adobe Reader)

ISBN-13 978-0-521-82157-5 hardback
ISBN-10 0-521-82157-6 hardback

Contents

Contributors

James R. Brown
Bioinformatics Division
Genetics Research
GlaxoSmithKline
1250 South Collegeville Road
UP1345
Collegeville, PA 19426
USA

Eric Déziel
INRS-Institut
Armand-Frappier
531 Boulevard des Prairies
Laval
Québec
Canada H7V 1B7

Irena Draskovic
Public Health Research Institute
225 Warren Street
Newark, NJ 07103
USA

David Dubnau
Public Health Research Institute
225 Warren Street
Newark, NJ 07103
USA

Laura S. Frost
Department of Biological Sciences
University of Alberta
Edmonton
Alberta
Canada T6G 2E9

Ian Grainge
Blanch Lane
South Mimms
Pottters Bar
Hertfordshire EN6 3LD
UK

Michael J. Gubbins
Department of Biological Sciences
University of Alberta
Edmonton
Alberta
Canada T6G 2E9

Jörg Hacker
Institut für molekulare
Infektionsbiologie
Universität Würzburg
Röntgenring 11
D-97070 Würzburg
Germany

Bianca Hochhut
Institut für molekulare Infektionsbiologie
Universität Würzburg
Röntgenring 11
D-97070 Würzburg
Germany

Diarmaid Hughes
Department of Cell and Molecular Biology
Box 596
Biomedical Center
Uppsala University
S-751 24 Uppsala
Sweden

Makkuni Jayaram
Section of Molecular Genetics and Microbiology
University of Texas at Austin
USA

Peter Mullany
Division of Microbial Diseases
Eastman Dental Institute for Oral Health Care Sciences
University College London (UCL)
University of London
256 Gray's Inn Road
London, WC1X 8LD
UK

Tobias Norström
Department of Cell and Molecular Biology
Box 596
Biomedical Center
Uppsala University
S-751 24 Uppsala
Sweden

Marie-Agnès Petit
U571
Faculté de médecine Necker-Enfants Malades
156 rue de Vaugirard
75015 Paris
France

Adam P. Roberts
Division of Microbial Diseases
Eastman Dental Institute for Oral Health Care Sciences
University College London (UCL)
University of London
256 Gray's Inn Road
London, WC1X 8LD
UK

Sally J. Rowland
University of Glasgow
56 Dumbarton Road
Glasgow G11 6NU
Scotland
UK

W. Marshall Stark
University of Glasgow
56 Dumbarton Road
Glasgow G11 6NU
Scotland
UK

Richard Villemur
INRS-Institut
Armand-Frappier
531 Boulevard des Prairies
Laval
Québec
Canada H7V 1B7

William R. Will
Department of Biological Sciences
University of Alberta
Edmonton
Alberta
Canada T6G 2E9

Steven Zimmerly
Department of Biological Sciences
University of Calgary
2500 University Drive NW
Calgary
Canada AB T2N 1N4

Part 1 Basic Mechanisms of Genome Rearrangement in Bacteria

CHAPTER 1

Mechanisms of homologous recombination in bacteria

Marie-Agnès Petit

Homologous recombination promotes the pairing between identical – or nearly identical – DNA sequences and the subsequent exchange of genetic material between them. It is an important and widely conserved function in living organisms, from bacteria to humans, that serves to repair double-stranded breaks or single-stranded gaps in the DNA, arising as a consequence of ionizing radiations, ultraviolet (UV) light, or chemical treatments creating replication-blocking adducts (Kuzminov, 1999). More recently, homologous recombination functions were also found in bacteria to rescue replication forks that have stalled for various reasons, such as a missing factor (e.g., the helicase), or a particular difficulty upstream of the fork, such as supercoiling or intense traffic of proteins (Michel et al., 2001).

Besides its molecular role, homologous recombination has played a major role in genome dynamics, by changing gene copy numbers through deletions, duplications, and amplifications: Intrachromosomal recombination between ribosomal operons or between mobile elements scattered into the genome leads to deletion or tandem duplications of large regions within the genome, up to several hundred kilobases (Roth et al., 1996). The duplications are unstable. Mostly they recombine back to the parental organization, and, therefore, remain undetected, except when appropriate selection, by gene dosage mostly, is exerted (Petes and Hill, 1988). In contrast, such duplications are ideal substrate for the diversification of genes: One gene is kept intact whereas the other is mutagenized, which leads to the birth of gene families. Once the duplicated segment has sufficiently diverged, it becomes more stable because of the lack of perfect homology to recombine the duplicated segment. Tandem duplications are also the starting point for further gene amplification, the repetition up to 20- to 100-fold of the tandem array (Kodama et al., 2002).

Finally, homologous recombination is also critical in terms of evolution, by allowing the generation of new allele combinations, and, as a consequence, the possibility to evolve and adapt to new environments, a hallmark of living organisms. In eukaryotes this happens mainly through meiosis, whereas in bacteria and archea the so-called "horizontal transfer" of genes is taking place on a larger scale (Ochman, Lawrence, and Groisman, 2000). Homologous recombination is one of the mechanisms through which such gene transfers occur, in particular during generalized transduction, conjugation, and natural transformation.

Much of what is known at the molecular level about homologous recombination in bacteria is from the in-depth work realized over the last 50 years on *Escherichia coli* (*E. coli*). For more recent reviews on this topic, the reader is referred to Kuzminov (1999) and Cox (2001). This chapter begins with a brief description of the knowledge based on the *E. coli* paradigm, but its main focus is on how other eubacteria resemble or differ from the paradigm. Because this book is on genomic rearrangements, plasmid recombination, which is a field in itself, is excluded.

HOMOLOGOUS RECOMBINATION: THE DNA ACTORS

Toward a definition of homologous recombination

The more processes of homologous recombination are known at the molecular level, the more difficult they are to be defined precisely. Concerning the DNA partners, in the original definition, homologous recombination concerned only events between pairs of chromosome homologs, and, therefore, was restricted to diploid cells. It then appeared that recombination could also concern two sequences at different loci (either in the same or in different chromosomes), the so-called "ectopic recombination." Finally, and especially in bacteria, homologous recombination was found to be a major way to integrate incoming DNA into a genome.

At the molecular level, homologous pairing and strand exchange may occur between two DNA molecules without any consequence at the genetic level, the so-called "non–cross-over" products (see the section "The DNA intermediates" in this chapter). The process is silent phenotypically, but essential molecularly, as it leads to DNA repair. During such a process, however, some point mutations of one molecule engaged into the homologous pairing may be transferred to the other molecule, and lead to gene conversion. This is not only frequent in fungi but also takes place in bacteria (Abdulkarim and Hughes, 1996). Finally, the enzymes that process homologous recombination

intermediates are able to pair sequences that are not identical, but partially diverged or homeologous (see the following section "The DNA products"). To summarize, homologous recombination does not necessarily recombine DNA and does not necessarily involve chromosome homologs, or identical sequences. In bacteria, one may define homologous recombination as all *recA*-dependent events, but even this simple assessment is not always true (see "RecA-independent homologous recombination" in this chapter).

The DNA products

Homologous recombination has its primary consequence at the DNA level; therefore, the main products of the process are first described. Most bacterial genomes are circular, and recombination products leading to linear chromosomes are lethal, so the focus is on circular products.

When the bacterial chromosome recombines with incoming DNA, as is the case during horizontal transfer, two main products are expected: If the entering DNA molecule contains two different stretches of homology with the bacterial chromosome (Fig. 1.1A), recombination can proceed by "double cross-over" (DCO). In this case, the intervening part of the chromosome is exchanged with the incoming DNA, and, therefore, lost. The DCO product is stable because no repeated sequences flank the incoming DNA. If the entering molecule contains a single stretch of homologous DNA and is circular – for instance, a nonreplicative plasmid – recombination occurs by single crossing over (sometimes called "Campbell-type" recombination, Fig. 1.1B). It produces a recombined chromosome in which the incoming

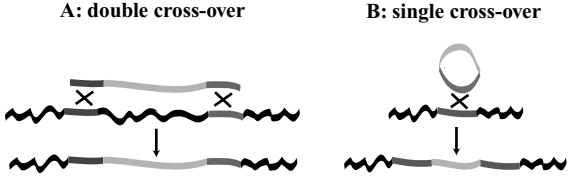


Figure 1.1. Two examples of integration of incoming DNA (flat lines, grey color) into the bacterial chromosome (black wavy lines). **(A)** The incoming DNA is linear and contains two regions of homology (dark grey) with the chromosome, each recombines (between the regions shown as a cross), and the resulting recombinant has integrated the foreign DNA by double cross-over. **(B)** The incoming DNA is circular and contains one region of homology (dark grey) with the chromosome. Upon recombination by single cross-over (shown as a cross), the foreign DNA is flanked by two copies of the homologous region, oriented in parallel.

DNA is flanked by directly repeated sequences, sometimes called "pop-in" recombinant. No DNA has been lost, but the resulting recombinant is unstable because it can "pop out" by homologous recombination. In cases where the introduced DNA confers a selective advantage, such recombinants are maintained. This process is widely used by geneticists to interrupt genes in bacteria such as *Bacillus subtilis*, *Lactococcus lactis*, *Deinococcus radiodurans*, and so on.

Chromosomal DNA can also generate intramolecular recombinants. This happens due to recombination between members of a gene family dispersed in the genome, typically between *rrn* operons or between mobile elements. If the two identical copies are inverted with respect to one another, the product is an inversion of the intervening sequence. If the two copies are oriented the same way, the process is called unequal crossing over because recombination probably takes place in an "unequal way" between sister chromatids behind a replication fork (Fig. 1.2). It leads to one chromosome containing a duplicated stretch flanked by the sequences that served to initiate the cross-over, and the other chromosome deleted for this same stretch of DNA, most likely unable to give a progeny. The chromosome containing the duplicated region is called a merodiploid or partial diploid; it is highly unstable and tends to recombine back to its original configuration. However, the frequency of production of these merodiploid is quite high: At any given time for one particular duplication, around 10^{-4} of an *E. coli* K12 population

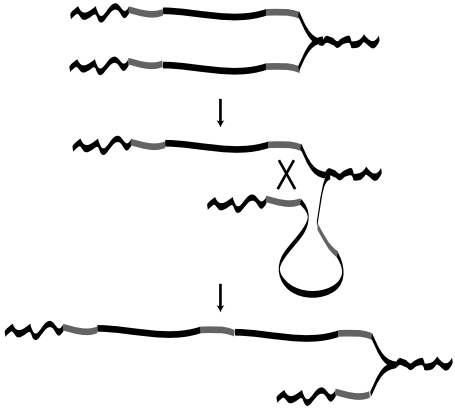


Figure 1.2. Intrachromosomal recombination. If two identical copies of a gene or a mobile element (grey and flat lines) are present on a chromosome (black wavy lines), they can recombine together (shown as cross) behind a replication fork to produce one chromosome with a deletion of the intervening region, and the other with a duplicated region.

is in the merodiploid state (Anderson and Roth, 1977; Petes and Hill, 1988). Such rearrangements offer a chance for the selection of new chromosomal variants and evolution.

The DNA intermediates

It is now generally admitted that a free DNA end, either a double-stranded extremity or a single-stranded gap, is the prerequisite to initiate homologous recombination. Two models based on this assumption and adapted from the review of Kuzminov (1999) are presented in Fig. 1.3. One starts with a double-stranded extremity (Fig. 1.3A), and the other starts with the single-stranded gap (Fig. 1.3B). In both cases, the initial event and the final event are the

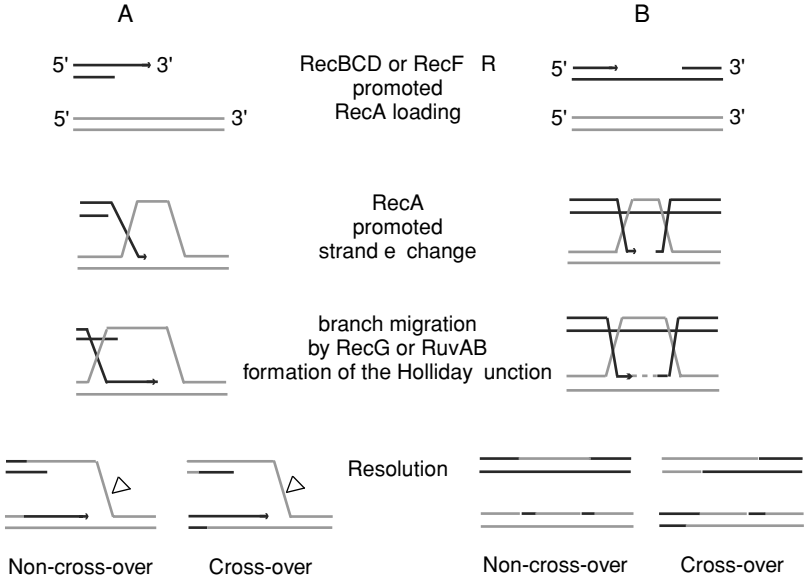


Figure 1.3. Two models showing possible intermediates of the recombination process, as adapted from Kuzminov (1999). Recombination starts from a double-stranded break (column A) or a single-stranded gap (column B) blue molecule, which is processed by RecBCD (left) or RecFOR (right) to load RecA and promote strand pairing with an intact grey molecule (step 2). Each strand of the DNA duplex is drawn, and 5′ and 3′ extremities are indicated on the first lanes. The black arrow shows the 3′ extremity of an invading molecule. Due to the action of the RecG and/or RuvAB helicase, Holliday junctions are created (step 3) and resolved by RuvC or another nuclease into crossed-over or non–crossed-over products (step 4). Dotted lane indicates DNA synthesis, and the white triangle stands for a putative nuclease that would cleave the D loop.

most documented ones, whereas the steps in between are speculative – in particular, the short patch of DNA synthesis by unknown polymerase and the cleavages by unknown endonuclease. Initial to all homologous recombination is the invasion of a single-stranded DNA into a duplex molecule so it pairs and forms the so-called "heteroduplex." Next – and less well characterized – this intermediate is converted into a Holliday junction (HJ), in which the second strand of the invading molecule has also paired with its recipient. This four-stranded structure is able to move around (branch migration) and then to be processed by a specific nuclease into either of two products, depending on the orientation of the cleavage. One cleavage will result in product molecules having received a small patch of the donor DNA, the heteroduplex region; it is called *gene conversion*. The other cleavage orientation will result in the exchange of the flanking sequences, or "crossing over," between the two recombining molecules.

The third model (Fig. 1.4) accounts for the repair of blocked replication fork (Seigneur et al., 1998). Recombination genes are not essential in bacteria (bacteria deleted for *rec* genes are viable), but they can become essential for the viability of replication mutants. In such mutants, replication fork progression is hindered, and a process called *replication fork reversal* is supposed to take place, in which the DNA intermediate is structurally identical to the HJ (Fig. 1.4B). This intermediate is processed by recombination enzymes and rescues the replication fork by removing the replication block either by recombination (Fig. 1.4E) or by trimming the extremity of the new strands (Fig. 1.4D) to allow restart. If the recombination enzyme RecBCD (see Chapter 2) does not process this intermediate, it is subjected to a cleavage by RuvABC (see Chapter 2), which may be lethal (Fig. 1.4C). The little revolution brought about by this model is that recombination enzymes reveal themselves as being closely interconnected with the replication process and more generally involved in the normal life cycle of a bacterium, rather than being specialized in some aspects of lesion repairs, or even more specialized processes, such as conjugation or transformation.

GENETICS AND BIOCHEMISTRY OF HOMOLOGOUS RECOMBINATION IN *E. COLI*

A wealth of genes and proteins play a role in homologous recombination processes in *E. coli*. They are briefly listed, and refer to the recombination models described in Fig. 1.3. Three distinct and successive steps are involved in homologous recombination: presynapsis, during which enzymes prepare the DNA substrate for RecA; synapsis, where RecA bound to DNA promotes

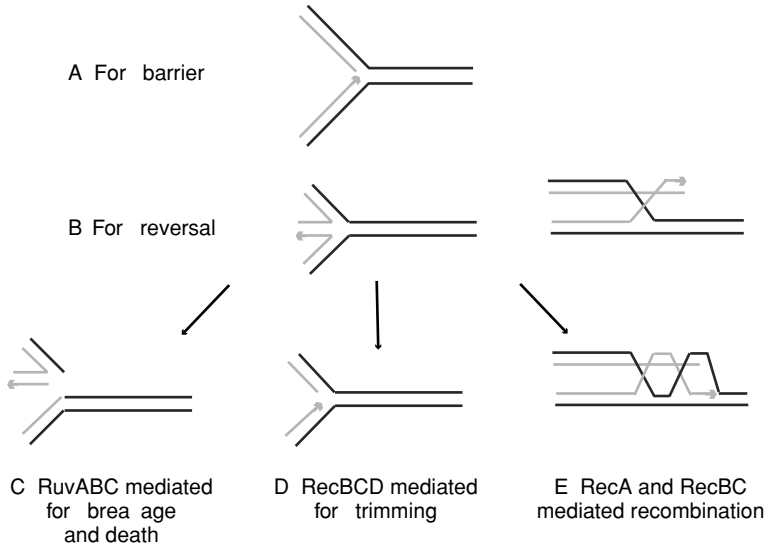


Figure 1.4. A model for the repair of a blocked replication fork by recombination enzymes, adapted from Seigneur et al. (1998). A replication fork is drawn with newly replicated strands in a light grey color, the arrow showing its 3′ extremity. The question mark stands for the block, which leads to a fork reversal, where the two replicated strands have paired (step B). This intermediate can be drawn such that the newly paired region faces its unreplicated homolog, ahead of the reversed fork (step B, right part). Three possible fates for this intermediate have been proposed: breakage if RuvABC arrives on the DNA before RecBCD (step C), fork trimming if RecBCD acts on the exposed double-strand end (step D), and recombination if RecBCD and RecA act in concert (step E). For simplicity the reversed fork is shown with blunt extremities, but a normal fork would tend to produce a 3′ protruding end because of the advance of the leading strand relative to the lagging strand. Such an extremity may be processed by exonucleases to produce a blunt end or be used directly by RecA in step E.

the active search for a homologous molecule; and postsynapsis, where intermediates are processed into products.

Presynapsis

Two sets of proteins are needed in *E. coli* for the processing of DNA ends and the efficient loading of RecA:

- The RecBCD heterotrimer is a dual enzyme that is very well characterized biochemically (Kowalczykowski et al., 1994; Myers and Stahl, 1994) and structurally (Singleton et al., 2004). It is a potent

exonuclease (ExoV) strictly dependent on blunt or nearly blunt ends for its activity, as well as a highly processive helicase. The RecB subunit encodes the nuclease activity, which degrades preferentially in the 3′ to 5′ direction, and the RecD subunit modulates RecB: Upon interaction with a specific DNA sequence, the Chi site, RecD converts the polarity of DNA degradation by RecB, such that a 3′ single-stranded extremity is created, and this promotes the loading of RecA (Anderson and Kowalczykowski, 1997). In addition, both RecB and RecD act as DNA helicases, of opposite polarity and different speed (Dillingham et al., 2003; Taylor and Smith, 2003). RecC is inert enzymatically, it serves as a structural component allowing the physical separation of the two DNA strands, and it is proposed to contain the Chi recognition site (Singleton et al., 2004). How exactly the RecBCD complex recognizes the Chi site is not yet understood. *recB* and *recC* mutants are sensitive to UV and gamma irradiation, and deficient for homologous recombination when the DNA substrate has a double-stranded blunt end, such as during generalized transduction and conjugation (Fig. 1.3A). Interestingly, *recB* or *recC* mutations profoundly affect cell growth, with up to 80% of dead cells in a liquid culture (Capaldo, Ramsey, and Barbour, 1974), and tend to yield suppressor mutations. Such is not the case for *recA* mutants and points to an additional role of the RecBCD complex in the cell, besides recombination, probably its exonucleolytic role, removing useless – or potentially dangerous – linear DNA. The strict dependence of the RecBCD complex for double-stranded ends makes this complex the first actor to act on linear DNA (Fig. 1.3, left side), and one of the key enzymes for the rescue of arrested replication forks (Figs. 1.4D and 1.4E) and for preventing RuvABC mediated fork breakage (Fig. 1.4C).

- The second group of proteins comprises RecF, RecO, and RecR. Although less well characterized at the molecular level, RecF, RecO, and RecR play a key role in preparing substrates for RecA on gapped DNA (Fig. 1.3, right side), to which RecBCD has no access (Morimatsu and Kowalczykowski, 2003). Their role would consist of competing away the single-stranded binding protein SSB to favor RecA loading. In addition to this role, RecFOR may also process double-strand breaks in cells in which the RecBCD complex is absent (Amundsen and Smith, 2003). For this purpose, at least two additional functions are recruited, the RecJ 5′ to 3′ nuclease, and the RecQ helicase (or, in its absence, UvrD or Helicase IV). These accessory functions would serve to trim the blunt extremity into a 3′ single strand for RecA. More recently, it was shown that RecFOR

and RecJ could collaborate with RecBCD when the RecB component has lost its nuclease activity (Ivancic-Bace et al., 2003). *recF*, *recO*, and *recR* mutants are less sensitive than *recB* or *recC* mutants to radiation, but double mutants *recB recF* are nearly as sensitive as the *recA* mutant strain. *recF*, *recO*, or *recR* mutants do not exhibit any particular growth defects. This may reflect the low frequency of gap formation, the exclusive RecFOR substrate, in *E. coli*.

Synapsis

The RecA protein forms a stable filament on single-stranded DNA (ss DNA), which extends in the 5′ to 3′ direction (Roca and Cox, 1997). It promotes ss DNA pairing with a homologous double-stranded DNA, which is the key step of homologous recombination. Once the two DNA molecules have been placed in register, strand exchange can start. Homologous recombination is completely abolished in *recA* mutants, except in two particular cases mentioned in the section "RecA-independent homologous recombination." *recA* mutants are highly sensitive to UV and gamma radiation, and also affected for their growth, with a reduced doubling time and 50% of dead cells in liquid culture (Capaldo, Ramsey, and Barbour, 1974).

The minimal length of DNA on which RecA binds *in vitro* is 8nt, and the smaller stable duplex made by RecA is 15 base pair (bp) long (Hsieh et al., 1992). *In vivo*, the minimal length of homology on which RecA can act is probably as small as 23 to 27 bp, but data vary according to the system used (Lloyd and Low, 1996). RecA also promotes strand exchange between sequences that are not identical *in vitro*, and *in vivo*, an editing process mediated by MutS and MutL aborts such intermediates by a mechanism that remains to be elucidated. As a consequence, genetic exchanges between closely related species with nearly identical DNA sequence (called homeologous sequence, diverged up to 15% or even more) are highly increased when the recipient strain is mutated for the *mutS* or *mutL* function (Rayssiguier, Thaler, and Radman, 1989; Vulic et al., 1997). MutS and MutL were also found to edit intrachromosomal rearrangements between two slightly diverged *rhs* sequences (Petit et al., 1991). A more recent study on a wide spectrum of natural *E. coli* isolates has revealed that such mutant strains are present in 3% to 5% of isolates (Denamur et al., 2002), and they should favor horizontal gene transfer between related species.

In addition to its role at the heart of recombination, RecA of *E. coli* is also responsible for inducing the SOS response (see "The SOS response"),

and for promoting the first step of replication fork reversal in some cases (McGlynn and Lloyd, 2000; Robu, Inman, and Cox, 2001; Seigneur, Ehrlich, and Michel, 2000).

Postsynapsis

Downstream of the RecA-promoted strand exchange, two alternative enzymes process the intermediates, RuvABC and RecG (Sharples, Ingleston, and Lloyd, 1999). RuvA is a DNA-binding protein specific for HJs, and RuvB is a DNA helicase that catalyzes branch migration when bound to RuvA. This helicase promotes the branch migration of HJ and delivers them to the specific nuclease RuvC, which resolves the recombination intermediate by cleaving symmetrically across the junction. Depending on which strands are cleaved, different products are expected, as drawn on Figs. 1.3A and 1.3B. RecG is also a DNA helicase, which favors branch migration of HJ and three-stranded branched structures (Lloyd and Sharples, 1993). Whether a nuclease is also involved to cleave the junctions processed by RecG is not known at present. What prompted the conclusion that *ruvABC* and *recG* encode redundant functions was the genetic observation that single mutants were only partially affected for recombination and partially sensitive to UV radiation, whereas the double mutant was as deficient and as sensitive as a *recA* strain, and affected for viability (Lloyd, 1991).

The outcome of recombination events appears to differ markedly with each situation, the RuvABC complex favoring the cross-over products when recombination is initiated from a double-stranded break, and the non–cross-over products when recombination is initiated from a gap (Cromie and Leach, 2000). Concerning RecG, one study suggests that it favors the cross-overs when recombination is initiated from a gap (Michel et al., 2000).

During the replication fork repair process (Fig. 1.4), a toxic role of RuvABC has been revealed: It recognizes the putative regressed fork intermediate, which has an HJ structure, and cleaves it, which leads to a linear chromosome. This aberrant role is countered by RecBCD, which either degrades the tail or initiates recombination with RecA (Seigneur et al., 1998).

Involvement of DNA replication

Some recombination intermediates (e.g., the one drawn in Fig. 1.3, last step, left side) are converted into replication forks due to the action of a group of seven proteins called collectively the PriA-dependant primosome, composed of PriA, PriB, PriC, DnaT, DnaC, DnaB, and DnaG (Marians, 1999,

2000). These proteins allow the loading of the replicative helicase, DnaB, and the DNA primase, DnaG, which are both absolutely required for starting coordinated replication of two DNA strands. The importance of this replication step during homologous recombination has been underlined by the observation that *priA* mutants are defective for conjugation and generalized transduction (Kogoma et al., 1996). In addition, *priA* mutants exhibit extremely slow growth and readily produce suppressor mutations, a phenotype more severe than *recA*, and even *recB* or *recC* mutants, which suggests that PriA plays an additional role elsewhere in the cell. A simple possibility is the replication restart at forks that lost the DnaB helicase. Once the DnaB and DnaG proteins are loaded, the DNA polymerase III holoenzyme takes over the replication step (Xu and Marians, 2003), and nothing is known at present concerning where and how replication stops.

Distinct from this main involvement of DNA replication during "ends-out" recombination, some short patches of DNA synthesis may also be needed for recombination starting from a gap, as shown on the right part of Fig. 1.3. The enzymes involved in this short DNA synthesis remain to be identified.

The SOS response

When *E. coli* cells are stressed by exposure to UV, gamma radiations, or chemical agents cross-linking the DNA, the SOS response, or SOS regulon, is induced. It consists of set of 31 (Fernandez De Henestrosa et al., 2000) to 50 genes (Courcelle et al., 2001), half of unknown functions, whose transcription is under the control of the LexA repressor. This repressor efficiently autocleaves into its inactive form when it contacts the RecA nucleofilament bound to DNA. RecA acts as a co-protease of LexA and this form of RecA is referred to as "RecA star." In this elegant way, once the cell senses DNA damage, by way of the RecA bound to a single-stranded portion of the DNA, the SOS genes are derepressed, among which four are directly involved in homologous recombination: RecA, present normally at 8000 molecules per cell is induced 10-fold, RuvA and RuvB are induced 2- to 3-fold, and RecN is induced 20-fold. Among the unknown SOS functions, one may encode a factor inhibiting the nuclease activity of RecBCD, and, therefore, favor its recombination activity (Rinken and Wackernagel, 1992). Once the recombination process is over and RecA has left the DNA, the LexA protein, which is itself induced by SOS, is no longer cleaved, and repression resumes. The DinI protein may also contribute to the closing off of the response by inhibiting the co-protease activity of RecA (Voloshin et al., 2001).

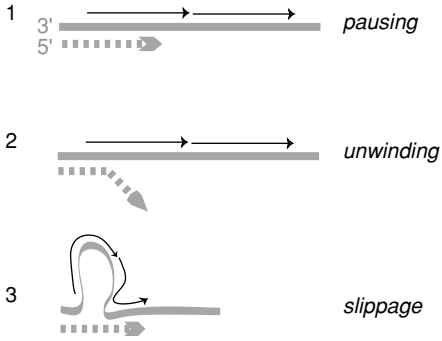


Figure 1.5. A model for recombination by replication slippage between tandem repeats, adapted from Viguera, Canceill, and Ehrlich (2001). Slippage is proposed to occur while a DNA polymerase replicates single-stranded DNA (shown as a thick grey line), as present on the lagging strand of a replication fork. If the DNA polymerase pauses in a region of tandem repeats (indicated with black arrows over the single-stranded DNA), the newly replicated strand (dotted thick grey line) may unwind (step 2) and pair erroneously with the downstream repetition (step 3). Then replication resumes, and the heteroduplex molecule leads after one more round of replication to a chromosome with a deletion of one repetition.

RecA-independent homologous recombination

Two cases of RecA-independent homologous recombination are described in *E. coli*. The first concerns recombination between short (around 1 kb long) tandem repeats, which recombine approximately as efficiently via a RecA-dependent process and a RecA-free process (Bierne et al., 1997; Saveson and Lovett, 1997). This RecA-free process is believed to result from replication slippage (Fig. 1.5) of the DNA polymerase when the tandem region is replicated: After a replication pause, the tip of the newly synthetized strand may be unwound and reanneal erroneously with the downstream copy of the tandem (Canceill and Ehrlich, 1996; d'Alencon et al., 1994; Viguera, Canceill, and Ehrlich, 2001). The close proximity between the recombining sequences is probably a strict requirement for such a recombination, and it may explain why such tandem repeats are rare in bacterial genomes.

The second case in which *E. coli* recombines its homologous DNA without using RecA concerns the activation of two cryptic genes – encoded by the Rac prophage, *recE* and *recT* – by the *sbcA* mutation that turns on the transcription of the operon. These functions are mentioned at the end of the next paragraph.

HOMOLOGOUS RECOMBINATION IN BACTERIA OTHER THAN *E. COLI* AND IN BACTERIOPHAGES

Bacteria are famous for their ability to conquer all kinds of habitats, as well as to live in highly complex ecosystems. As recombination appears more intrinsically linked with the everyday life of bacteria, it is of a high interest to survey how far the functions uncovered for *E. coli* are relevant or adapted to each species. For instance, a pathogen such as *Helicobacter pylori* has a huge propensity to mutate and rearrange its genome, and this may correlate to its pathogenicity (Loughlin et al., 2003). Also, the invasive *Salmonella typhimurium* pathogen appears to rely strongly on recombination functions to overcome the stresses endured on entry into macrophages (Schapiro, Libby, and Fang, 2003). The increasing number of fully sequenced genomes, as well as the powerful bioinformatic tools available today will allow a general overview over 50 eubacterial genomes (Table 1.1). The updated version of COG (cluster of orthologous groups, http://www.ncbi.nlm.nih.gov/COG/) has been used for this purpose (Tatusov, Koonin, and Lipman, 1997; Tatusov et al., 2001). Bacteriophages, especially when they are temperate or remain as remnants in bacterial genomes, are able to contribute to homologous recombination in bacteria and are briefly mentioned at the end of this section. Archea, although sharing ecological niches with bacteria and exchanging DNA with them through horizontal transfer, were not included in this study because they encode eukaryotic-like proteins with respect to homologous recombination, a field beyond the scope of this chapter.

The RecBCD and AddAB classes of exonucleases/helicases

S. typhimurium recBCD genes are essential for its infectivity (Buchmeier et al., 1993). This function is apparently needed to resist the stress due to nitric oxide (NO) encountered in macrophages, and a nice set of genetic evidence supports the model of recombination depicted in Fig. 1.4, where the consequence of NO would be to provoke replication fork arrests and a RecG-dependent reversal. Unless protected by RecBCD, this reversed fork is subjected to RuvC-dependent cleavage and subsequent death (Schapiro, Libby, and Fang, 2003).

A functional analog of RecBCD, called AddAB, has been characterized genetically and biochemically in *Bacillus subtilis*, a gram-positive bacterium isolated from soil, which is naturally competent (Chedin and Kowalczykowski, 2002). AddA and RecB share 21% identical amino acids, but AddB and RecC

Table 1.1. *Distribution of recombination functions among 50 bacteria*

Group	High GC Gram+			Low GC Gram +										"Ancient" bacteria					
	Actinobact.			bacilli / clostridia						parasites									
Name	Cgl	Mtu	Mle	Cac	Lla	Spy	Sau	Lin	Bsu	Uur	Mpu	Mpn	Mge	Tma	Fnu	Aae	Syn	Nos	Dra
O.P. or O.E(a)		*	*			*				*	*	*	*						
RecB		■																	
AddA				■	■	■	■	■	■						■				
RecR	■	■	■	■	■	■	■	■	■	■	■				■	■	■	■	■
RecO	■	■	■	■	■	■	■	■	■	■	■				■		■	■	■
RecF	■	■	■	■	■	■	■	■	■	■	■				■		■	■	■
RecJ	■			■	■	■	■	■	■					■	■	■	■	■	■
RecQ	■			■	■		■	■	■						■		■	■	■
RecN	■	■	■	■	■	■	■	■	■						■	■	■	■	■
RecA	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
RuvA	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■
RuvB	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■
RuvC	■	■	■											■	■		■	■	■
RecU					■	■	■	■	■	■	■	■	■						
RecG	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■
LexA	■	■	■	■	■		■	■	■					■			■	■	■
PriA	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■
PriB																			
PriC																			
DnaD				■	■	■	■	■	■										
DnaB					■	■	■	■	■										

Group	Chlam & Spiro				Proteobacteria																	
					δ / ε		α						β		γ							
Name	Ctr	Cpn	Tpa	Bbu	Hpy	Cje	Atu	Sme	Bme	Mlo	Ccr	Rpr	Rso	Nme	Ec	Ype	Sty	Vch	Pae	Hi	Xfa	Bu
O.P. or O.E(a)	*	*	*	*								*								*		*
RecB		■		■										■	■	■	■	■	■	■	■	■
AddA	■		■		■	■	■	■	■	■	■	■	■									
RecR	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
RecO	■	■	■				■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
RecF	■		■				■	■	■	■	■	■			■	■	■	■	■	■	■	
RecJ	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
RecQ			■				■	■		■	■		■	■	■	■	■	■	■	■	■	
RecN			■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
RecA	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
RuvA	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
RuvB	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
RuvC	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
RecU																						
RecG			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
LexA							■	■	■	■	■		■		■	■	■	■	■	■	■	
PriA	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
PriB													■	■	■	■	■	■	■	■	■	
PriC															■	■	■	■	■	■	■	
DnaD																						
DnaB																						

(a) O.P. obligate parasit, O.E. obligate endosymbiont

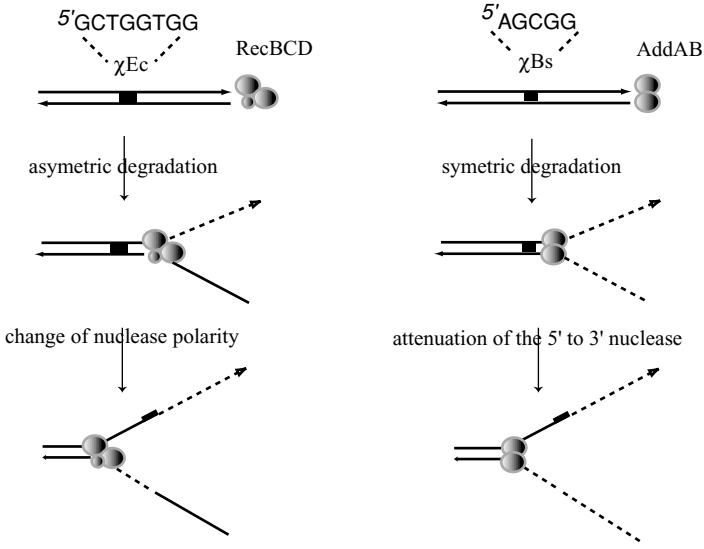


Figure 1.6. A summary of the biochemical properties of RecBCD of *E. coli* and AddAB of *B. subtilis*, adapted from Chedin and Kowalczykowski (2002). The two strands of a DNA molecule are drawn, with an arrow showing the 3′ extremities. The black rectangle stands for the Chi site, and dotted lines for the DNA degraded by nucleases. The main difference between the two sets of enzymes is visible in step 2: Degradation is asymmetric for RecBCD, and symmetric for AddAB. See text for more details.

are unrelated. Even if a *B. subtilis* ORF aligns with *E. coli recD*, it seems unrelated to the activity of the AddAB enzyme (Chedin and Kowalczykowski, 2002). Both AddA and AddB contain nuclease motives (Quiberoni et al., 2001), and it is supposed that they degrade strands of opposite polarity. Upon encounter of a specific sequence, different from the *E. coli* one but also called a Chi site, AddA, but not AddB, stops degrading DNA so that a 3′ single-stranded extremity is exposed (Chedin, Ehrlich, and Kowalczykowski, 2000). Whether this extremity facilitates RecA loading remains to be shown. A summary of the compared activities of RecBCD and AddAB is presented in Fig. 1.6. It is remarkable how similarly both enzymes work to achieve their role of nuclease/recombinase, despite their low sequence similarity.

The *addA* or *addB* mutants are affected for growth, with 80% of the cells in a liquid culture unable to form colonies, but do not yield suppressor mutations as *recBC* mutants. Interestingly, *add* mutants are less sensitive to UV radiations compared with *E. coli recBC* mutants (M.-A. Petit, personal

Table 1.2. *Sequence of the Chi site in various bacteria*

Organism	Sequence
E. coli	5′-GCTGGTGG-3′
H. influenzae	5′-GNTGGTGG-3′
B. subtilis	5′-AGCGG-3′
L. lactis	5′-GCGCGTG-3′

observation, 2003), and recombination during transformation is almost unaffected (Fernandez, Ayora, and Alonso, 2000). AddAB is not induced upon competence induction in *B. subtilis* (Ogura et al., 2002). Finally, when expressed in *E. coli*, AddAB partially complements the defects of a *recBC* mutant (Kooistra, Haijema, and Venema, 1993). Similar phenotypes have been reported for *rexAB* mutants (orthologs of *addAB*) in *Lactococcus lactis* (el Karoui, Ehrlich, and Gruss, 1998) and *Streptococcus pneumoniae* (Halpern et al., 2004).

Critical to the action of these nucleases/helicases is the recognition of a DNA sequence, the Chi site, which converts the nuclease into a recombinase. Interestingly, in all bacteria where it was looked for, the Chi sequence differs (Table 1.2). This has been proposed as a way of self-recognition for a bacterial species: Incoming DNA would be readily recombined into the genome if it contains Chi sites, and would be degraded otherwise (el Karoui et al., 1999).

Among fully sequenced bacterial genomes, three categories can be made, with respect to the presence of such a nuclease/recombinase: Those containing the RecBCD orthologs, those containing the AddA ortholog, and those free of any of these two functions (Table 1.3). Interestingly, no clear division among bacterial branches appears: Proteobacteria gamma and most beta contain RecBCD, whereas alpha and epsilon contain AddA. Among gram-positive bacteria, most contain the AddA enzyme, but actinobacteria contain the RecBCD version (or no version at all). The two sequenced spirochetes contain one enzyme type each. Whether bacteria free of RecBCD or the AddA counterpart encode a functional analog remains to be investigated. *Deinococcus radiodurans*, one of the most recombination-proficient bacteria, belongs to this group. During this search, it was observed that *recC*, *recB*, and *recD* genes are often clustered in a single operon, as well as *addB* and *A*. Some species in which *addA* is not preceded by an *addB* ortholog (AddB-orphan species) instead encode at this position an ORF containing a *recB* exonuclease

Table 1.3. *Distribution of RecBCD and AddA among 50 eubacteria*

	Group I: RecBCD	Group II A: AddA and AddB	Group II B: AddA and COG2887	Group III: no RecBC or AddA ortholog
Genes order	recC recB recD 	addB addA 	cog addA 	
Species	Bbu* Buc Ctr Cpn Eco EcZ Ecs Hin Mtu MtC Nme NmA Pae Pmu Vch Sty Xfa Ype	Bha Bsu Cac Lla Lin Spy Spn Sau	Atu Bme Ccr Cje Fnu Hpy jHp Mlo Rso Rpr Rco Sme Tpa	Aae Cgl Dra Mle Mpu Mpn Mge Nos Syn Tma Uur
Number of species per category	18	8	13	11

* Bacterial species abbreviations: **Aae**, *Aquifex aeolicus*; **Atu**, *Agrobacterium tumefaciens*; **Bha**, *Bacillus halodurans*; **Bsu**, B*acillus subtilis*; **Bbu**, *Borrelia burgdorferi*; **Bme**, *Brucella melitensis*; **Buc**, *Buchneria*; **Cac**, *Clostridium acetobutylicum*; **Ccr**, *Caulobacter crescentus*; **Cgl**, *Corynebacterium glutamicum*; **Cje**, *Campylobacter jejuni*; **Cpn**, *Chlamydia pneumoniae*; **Ctr**, *Chlamydia trachomatis;* **Dra**, *Deinococcus radiodurans;* **Eco**, **EcZ** and **Ecs**, *Escherichia coli;* **Fnu**, *Fusobacterium nucleatum;* **Hin**, *Haemophilus influenzae;* **Hpy** and **jHp**, *Helicobacter pylori;* **Lin**, *Listeria inocua;* **Lla**, *Lactococcus lactis;* **Mge**, *Mycoplasma genitalium;* **Mle**, *Mycobacterium leprae;* **Mlo**, *Mesorhizobium loti;* **Mpn**, *Mycoplasma pneumoniae;* **Mpu**, *Mycoplasma pulmonis;* **Mtu** and **MtC**, *Mycobacterium tuberculosis;* **Nme** and **NmA**, *Neisseria meningitides;* **Nos**, *Nostoc;* **Pae**, *Pseudomonas aeruginosa;* **Pmu**, *Pasteurella multocida;* **Rso**, *Ralstonia solanacearum;* **Rpr**, *Rickettsia prowazekii;* **Rco**, *Rickettsia conorii;* **Sau**, *Staphylococcus aureus;* **Spy**, *Streptococcus pyogenes;* **Spn**, *Streptococcus pneumoniae;* **Sty**, *Salmonella typhimurium;* **Sme**, *Sinorhizobium meliloti;* **Syn**, *Synechocystis;* **Tma**, *Thermotoga maritime;* **Tpa**, *Treponema palladium;* **Uur**, *Ureaplasma urealyticum;* **Vch**, *Vibrio cholerae;* **Xfa**, *Xylella fastidiosa;* **Ype**, *Yersinia pestis*.

motif (COG number 2887). Such an ORF, if not always adjacent to *addA*, is present in all AddB-orphan strains. Whether this ORF encodes an *addB*-like function is an open question. In addition, a phylogenetic tree of AddA (as presented in the page of AddA orthologs, COG number 1074) shows that all AddA-orphan species form a group separate from the species containing both AddA and AddB. For these reasons, I propose to group such bacterial species into a subcategory IIB.

The RecFOR complex

Bona fide orthologs of RecF, RecO, and RecR have been described in *B. subtilis*. Mutants in each of these genes behave similarly and define an epistatic group, as in *E. coli*. Interestingly, a fourth gene, *recL*, belongs to this epistatic group, but its genetic locus remains to be found (Alonso, Luder, and Tailor, 1991). A striking difference between *E. coli* and *B. subtilis* concerns the relative contribution of *recBCD*/*addAB* and *recFOR* functions to the resistance to UV radiation or chemical agents: *B. subtilis recFOR* mutants are very sensitive to UV radiation, as much as *recBCD* mutants of *E. coli*, and conversely, as mentioned previously, *addAB* mutants are as modestly sensitive as *E. coli recFOR* mutants. However, mutants missing both the RecBCD/Add and RecFOR functions are affected to a comparable extent in both hosts, and as affected as *recA* mutants, which suggests the absence of a third presynaptic activity in both bacteria.

Interestingly, the RecFOR functions are more widely spread among fully sequenced bacterial genomes than the RecBCD/Add function. Only 5 among 50 genomes do not contain any ortholog of at least one of the three functions. Among them, four are obligate intracellular parasites or obligate endosymbionts (Table 1.1). In some cases, one or even two of the three genes are missing, which could reflect either a loss of selective advantage for the RecFOR function and/or some undetected additional function for each unit of the complex. The RecR unit is present in all 45 species, which might favor the second possibility. RecO is missing in 4 of these 45 species (in particular in the delta/epsilon subdivision of Proteobacteria) and RecF is missing in all RecO minus species, and also in 4 more (in particular, in the beta subdivision of Proteobacteria). The loss of RecF, RecO, or RecR may be correlated with the loss of gene conversion at rDNA loci, which is observed in obligate endosymbionts (Dale et al., 2003). Indeed, this loss of homologous recombination function may constitute a first step toward chromosomal degeneration.

The RecA protein

In all bacteria in which it has been investigated, RecA is the central protein of homologous recombination and DNA repair. The *recA* gene from one species often complements the defects of another species *recA* mutant, even as distantly related as *B. subtilis* and *E. coli* (de Vos, de Vries, and Venema, 1983). One remarkable exception is the *recA* gene from *H. pylori*, which is inactive in *E. coli*, and appears to require posttranslation modification for activity (Schmitt et al., 1995). The *Pseudomonas aeruginosa* RecA, when expressed in *E. coli*, is threefold more active than *E. coli* RecA (Baitin Zaitsev, and Lanzov, 2003). The regulation of *recA* gene expression varies almost with each species in which it has been studied. In naturally competent species such as *B. subtilis* and *S. pneumoniae*, the *recA* gene is induced upon competence induction (Lovett, Love, and Yasbin, 1989). The *H. influenzae recA* gene is not induced during competence, but its expression is controlled by both LexA and cyclic AMP receptor protein (Zulty and Barcak, 1993). Finally, the *recA* gene expression of *D. radiodurans* is controlled positively by IrrE (Earl et al., 2002). Biochemical studies have revealed that *D. radiodurans* RecA behaves very differently from the *E. coli* enzyme, as it has double-stranded DNA as its preferred substrate. However, later stages of strand exchange appear comparable (Kim and Cox, 2002). The RecA protein is remarkably conserved among bacteria, but it is not universal, as among the COG 50 genomes list, it lacks in the *Buchneria* species.

The editing of recombination between diverged sequences by the MutLS proteins differs markedly between bacterial species. Although very efficient in *E. coli* during conjugation, the HexAB proteins of *Streptococcus pneumoniae* (MutLS orthologs) have a moderate effect during natural transformation, due essentially to a limitation in amounts of proteins available (Humbert et al., 1995). The MutLS proteins of *B. subtilis* are almost inoperative during transformation (Majewski and Cohan, 1998). This aspect is further developed in Chapter 3.

The RuvABC complex and the RecG helicase

In *H. pylori*, a species exhibiting high levels of genetic diversity, RuvC appears critical for continued survival *in vivo* in the mouse stomach (Loughlin et al., 2003). Here, the role of homologous recombination may differ from the one played in *S. typhimurium*, where the important function for infectivity is *recBC*, and where indeed addition of the *ruvC* mutation in a *recBC* mutant

Table 1.4. *Components of PriA-dependent primosomes*

	E. coli	*B. subtilis*
Initiation	PriA	PriA
Intermediate proteins	PriB & PriC & DnaT	DnaD
Helicase loader	DnaC	DnaB & DnaI
Replicative helicase	DnaB	DnaC

suppresses the attenuation phenotype (Schapiro, Libby, and Fang, 2003). Although *Salmonella* may use recombination to repair a stalled replication fork, *H. pylori* may use it to recombine genes. Interestingly, although RuvA and RuvB are almost universal among eubacteria, the RuvC element, required to cleave the HJ, is absent in all low G+C gram-positive bacteria. Interestingly, the RecU protein of *B. subtilis* was reported to cleave HJ (Ayora et al., 2004), an ortholog present in five of the six species representing low GC gram-positive bacteria in Table 1.1, so that it may indeed constitute a functional analog of RuvC in this branch.

The *mmsA* gene of *S. pneumoniae* encodes an ortholog of RecG, which has also similar biochemical characteristics (Hedayati, Steffen, and Bryant, 2002). This function is required for efficient natural transformation (Martin et al., 1996). RecG is found in 43 of the 50 sequenced genomes, and absent mostly in obligate parasites. This underlines the important role of this helicase in bacteria, which is probably many-fold and still far from fully understood at present.

Primosomal proteins

The primosomal proteins of *B. subtilis* have been analyzed in detail. A PriA ortholog is present and active, and the *priA* mutant exhibits the same low viability and tendency to accumulate suppressor mutations as its *E. coli* counterpart (Polard et al., 2002). However, the next components of the primosomal "cascade," namely DnaD, DnaI, and DnaB, are unrelated to the *E. coli* ones (Bruand et al., 2001). They differ in number, structure, and sequence from the PriB, PriC, DnaT, and DnaC proteins, but correspond to functional analogs (Table 1.4): They are involved in *B. subtilis* as mediators between PriA and the replicative helicase (Marsin et al., 2001; Velten et al., 2003). A limited domain, centered on an ATP-binding site, is common to DnaC of *E.*

coli and DnaI of *B. subtilis*, which has led sometimes to their grouping (as done in COG).

The analysis of the 50 fully sequenced bacterial genomes suggests that PriA is ubiquitous in bacteria: All but 4 obligate parasits encode a PriA ortholog. Interestingly, none of the mediator primosomal proteins, acting after PriA to load the replicative helicase, are present in more than a few species, as if each branch had formed its own set of proteins (Table 1.1).

SOS response

A LexA ortholog, the negative regulator of SOS response, is found in many bacterial species. However, in the case of *D. radiodurans*, this protein is not the repressor of the SOS response, and RecA expression in particular does not depend on LexA (Narumi et al., 2001), but on a positive regulator. The precise set of genes induced by LexA has been determined in *B. subtilis* (Dubnau and Lovett, 2002). It comprises 21 genes, and among recombination proteins, RecA and RuvAB are induced, as in *E. coli*, but not the RecN protein.

Some other unknown recombination proteins

A study of DNA repair mutants affected in homologous recombination in *B. subtilis* has been systematically undertaken over the years by the group of Dr. Juan Alonso (Fernandez, Ayora, and Alonso, 2000). Apart from the AddAB and RecFLOR functions already mentioned, some other genes, which define new epistatic groups, have been described. The *ruvA*, *recU*, and *recD* genes form the group epsilon, which is likely to take over the postsynaptic step of recombination (Ayora et al., 2004). The most interesting epistatic groups are group gamma (*recH* and *recP* genes) and zeta (*recS gene*, a *recQ* ortholog). Some genes locations (*recH, P, D*), roles, and functions are not yet defined, but they may reveal new ways to process DNA for homologous recombination.

The phage versions of recombination genes

Forterre has proposed that plasmid or virus DNA informational proteins could sometimes displace cellular analogs (Forterre, 1999). This possibility, combined with the huge reservoir of genes probably at hand with viral genomes, renews the interest for the study of phage recombination genes, which can only be briefly mentioned here. The best studied functional analog of RecA is RecT, encoded by the *E. coli rac* prophage. The RecT enzyme promotes not only *in vitro* single-stranded annealing (Hall and Kolodner, 1994), but also single-stranded invasion into duplex DNA (Noirot and

Table 1.5. *Phage recombination genes*

Phage name	Lambda	rac	DLP12	T4	T7	SPP1
Exonuclease	Redα	RecE				G34.1P
Nuclease/helicase				Gp46&47		
RecFOR analog	Orf					
Recombinase& ATPase				UvsX		
Recombinase	Redβ	RecT				G35P
Resolvase	Rap		Rus	Gp49	Gp3	

Kolodner, 1998), and the intermediates of the strand exchange reaction look strikingly similar to those generated by RecA (Noirot et al., 2003). However, unlike RecA, it does not have an ATP hydrolysis activity. Together with RecE, its associated exonuclease of the 5′ to 3′ polarity (Muyrers et al., 2000), it promotes *in vivo* double-stranded break repair (Takahashi et al., 1993), and the recombination between short homologous sequences (Zhang et al., 1998). The *rac* prophage belongs to the same family as the temperate bacteriophage lambda, which encodes orthologs of RecT/RecE as Redβ and Redα, respectively. Lambda also encodes a RecBCD inhibitor, Gam, so cells harboring the three Lambda functions are now widely used to introduce PCR fragments flanked by short, 50 bp long, homologies into the *E. coli* chromosome (Datsenko and Wanner, 2000; Yu et al., 2000). Lambda also possesses a functional analog of RecFOR encoded by the *orf* gene (Sawitzke and Stahl, 1997). Finally, homologous recombination is intrinsically connected with the replication cycle of some lytic bacteriophages like T4 of *E. coli* (Bleuit et al., 2001) and SPP1 of *B. subtilis* (Ayora et al., 2002). The genome of bacteriophage T4 encodes a RecA analog, UvsX, which hydrolyses ATP, like RecA, and gp46 and 47 RecBCD analogs (Miller et al., 2003), and SPP1 encodes a G35P ATP-independent recombinase and a G34.1P nuclease (Ayora et al., 2002; Martinez, Alonso, and Ayora, personal communication).

Finally some phages or prophages encode nucleases able to cleave HJs: gp49 for T4, gp3 for T7, Rap for lambda, and RusA for the DLP12 prophage (Sharples, 2001). Most of them are cleaving various branched DNA substrates, except for RusA, whose activity is restricted to HJ (Bolt and Lloyd, 2002). A summary of phage recombination proteins is presented in Table 1.5. One remarkable property of all these phage functions is that they do not exhibit sequence similarity with bacterial analogs. This underlines the rich diversity

of bacteriophage "solutions" leading to the same set of functions, presynapsis, synapsis, and postsynapsis of homologous recombination, and the difficulty to predict them by sequence analysis.

CONCLUSION

Although bacteria have some common key functions such as RecA or PriA, they also each possess some level of diversity in other components. The main differences observed concern the RecBCD versus AddA helicase/nuclease, which are distributed among bacterial groups with no apparent logic. This activity seems absent from a substantial number of species, 11 among the 50 analyzed here, and in particular, in *D. radiodurans*. The second best studied set of proteins active at the presynapsis stage, RecFOR, appears more conserved, and also more active in bacteria other than *E. coli*. More generally, this study has shown a number of cases of functional analogs, and, therefore, convergent evolution (RecBCD and AddAB, RecA and RecT, PriB/PriC/DnaT/DnaC and DnaD/DnaI/DnaB, the various RuvC analogs in phages), and the list may grow in the future. Nevertheless, a big leap exists between these bacterial enzymes and the one at play in archea and eukaryots. Some attempts at proposing functional analogs have been made (Cromie, Connelly, and Leach, 2001). More recently, Rad52, Rad55, and Rad57 proteins were proposed to act in a way parallel to the RecFOR enzymes (Morimatsu and Kowalczykowski, 2003). Whether these proposals hold true will certainly be seen in the near future.

ACKNOWLEDGMENTS

I want to thank Juan Alonso, Meriem El Karoui, Jann Martinsohn, Ivan Matic, Bénédicte Michel, Xavier Veaute, and Marin Vulic for critical reading of this manuscript, and Emmanuelle Le Chatelier for her precious help with Table 1.1.

REFERENCES

Abdulkarim, F., and Hughes, D. (1996). Homologous recombination between the tuf genes of *Salmonella typhimurium*. *J Mol Biol*, **260**, 506–522.

Alonso, J.C., Luder, G., and Tailor, R.H. (1991). Characterization of *Bacillus subtilis* recombinational pathways. *J Bacteriol*, **173**, 3977–3980.

Amundsen, S.K., and Smith, G.R. (2003). Interchangeable parts of the *Escherichia coli* recombination machinery. *Cell*, **112**, 741–744.

Anderson, D.G., and Kowalczykowski, S.C. (1997). The translocating RecBCD enzyme stimulates recombination by directing RecA protein onto ssDNA in a chi-regulated manner. *Cell*, **90**, 77–86.

Anderson, R.P., and Roth, J.R. (1977). Tandem genetic duplications in phage and bacteria. *Annu Rev Microbiol*, **31**, 473–505.

Ayora, S., Missich, R., Mesa, P., Lurz, R., Yang, S., Egelman, E.H., and Alonso, J.C. (2002). Homologous-pairing activity of the Bacillus subtilis bacteriophage SPP1 replication protein G35P. *J Biol Chem*, **277**, 35969–35979.

Ayora, S., Carrasco, B., Doncel, E., Lurz, R., and Alonso, J.C. (2004). Bacillus subtilis RecU protein cleaves Holliday junctions and anneals single-stranded DNA. *Proc Natl Acad Sci U S A*, **101**, 452–457.

Baitin, D.M., Zaitsev, E.N., and Lanzov, V.A. (2003). Hyper-recombinogenic RecA protein from *Pseudomonas aeruginosa* with enhanced activity of its primary DNA binding site. *J Mol Biol*, **328**, 1–7.

Bierne, H., Seigneur, M., Ehrlich, S.D., and Michel, B. (1997). uvrD mutations enhance tandem repeat deletion in the *Escherichia coli* chromosome via SOS induction of the RecF recombination pathway. *Mol Microbiol*, **26**, 557–567.

Bleuit, J.S., Xu, H., Ma, Y., Wang, T., Liu, J., and Morrical, S.W. (2001). Mediator proteins orchestrate enzyme-ssDNA assembly during T4 recombination-dependent DNA replication and repair. *Proc Natl Acad Sci U S A*, **98**, 8298–8305.

Bolt, E.L., and Lloyd, R.G. (2002). Substrate specificity of RusA resolvase reveals the DNA structures targeted by RuvAB and RecG in vivo. *Mol Cell*, **10**, 187–198.

Bruand, C., Farache, M., McGovern, S., Ehrlich, S.D., and Polard, P. (2001). DnaB, DnaD and DnaI proteins are components of the *Bacillus subtilis* replication restart primosome. *Mol Microbiol*, **42**, 245–255.

Buchmeier, N.A., Lipps, C.J., So, M.Y., and Heffron, F. (1993). Recombination-deficient mutants of *Salmonella typhimurium* are avirulent and sensitive to the oxidative burst of macrophages. *Mol Microbiol*, **7**, 933–936.

Canceill, D., and Ehrlich, S.D. (1996). Copy-choice recombination mediated by DNA polymerase III holoenzyme from *Escherichia coli*. *Proc Natl Acad Sci U S A*, **93**, 6647–6652.

Capaldo, F.N., Ramsey, G., and Barbour, S.D. (1974). Analysis of the growth of recombination-deficient strains of *Escherichia coli* K-12. *J Bacteriol*, **118**, 242–249.

Chedin, F., Ehrlich, S.D., and Kowalczykowski, S.C. (2000). The *Bacillus subtilis* AddAB helicase/nuclease is regulated by its cognate Chi sequence in vitro. *J Mol Biol*, **298**, 7–20.

Chedin, F., and Kowalczykowski, S.C. (2002). A novel family of regulated helicases/nucleases from gram-positive bacteria: Insights into the initiation of DNA recombination. *Mol Microbiol*, **43**, 823–834.

Courcelle, J., Khodursky, A., Peter, B., Brown, P.O., and Hanawalt, P.C. (2001). Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient *Escherichia coli*. *Genetics*, **158**, 41–64.

Cox, M.M. (2001). Recombinational DNA repair of damaged replication forks in *Escherichia coli*: Questions. *Annu Rev Genet*, **35**, 53–82.

Cromie, G.A., Connelly, J.C., and Leach, D.R. (2001). Recombination at double-strand breaks and DNA ends: Conserved mechanisms from phage to humans. *Mol Cell*, **8**, 1163–1174.

Cromie, G.A., and Leach, D.R. (2000). Control of crossing over. *Mol Cell*, **6**, 815–826.

d'Alencon, E., Petranovic, M., Michel, B., Noirot, P., Aucouturier, A., Uzest, M., and Ehrlich, S.D. (1994). Copy-choice illegitimate DNA recombination revisited. *Embo J*, **13**, 2725–2734.

Dale, C., Wang, B., Moran, N., and Ochman, H. (2003). Loss of DNA recombinational repair enzymes in the initial stages of genome degeneration. *Mol Biol Evol*, **20**, 1188–1194.

Datsenko, K.A., and Wanner, B.L. (2000). One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci U S A*, **97**, 6640–6645.

de Vos, W.M., de Vries, S.C., and Venema, G. (1983). Cloning and expression of the *Escherichia coli* recA gene in *Bacillus subtilis*. *Gene*, **25**, 301–308.

Denamur, E., Bonacorsi, S., Giraud, A., Duriez, P., Hilali, F., Amorin, C., Bingen, E., et al. (2002). High frequency of mutator strains among human uropathogenic *Escherichia coli* isolates. *J Bacteriol*, **184**, 605–609.

Dillingham, M.S., Spies, M., and Kowalczykowski, S.C. (2003). RecBCD enzyme is a bipolar DNA helicase. *Nature*, **423**, 893–897.

Dubnau, D., and Lovett, C.M., Jr. (2002). "Transformation and recombination." In Sonenshein, A.L., Hoch, J.A., and Losick, R. (eds.), *Bacillus subtilis and its closest relatives. From genes to cells*. Washington, DC: ASM Press, pp. 453–471.

Earl, A.M., Mohundro, M.M., Mian, I.S., and Battista, J.R. (2002). The IrrE protein of *Deinococcus radiodurans* R1 is a novel regulator of recA expression. *J Bacteriol*, **184**, 6216–6224.

el Karoui, M., Biaudet, V., Schbath, S., and Gruss, A. (1999). Characteristics of Chi distribution on different bacterial genomes. *Res Microbiol*, **150**, 579–587.

el Karoui, M., Ehrlich, D., and Gruss, A. (1998). Identification of the lactococcal exonuclease/recombinase and its modulation by the putative Chi sequence. *Proc Natl Acad Sci U S A*, **95**, 626–631.

Fernandez, S., Ayora, S., and Alonso, J.C. (2000). *Bacillus subtilis* homologous recombination: genes and products. *Res Microbiol*, **151**, 481–486.

Fernandez De Henestrosa, A.R., Ogi, T., Aoyagi, S., Chafin, D., Hayes, J.J., Ohmori, H., and Woodgate, R. (2000). Identification of additional genes belonging to the LexA regulon in *Escherichia coli*. *Mol Microbiol*, **35**, 1560–1572.

Forterre (1999). Displacement of cellular proteins by functional analogues from plasmids or viruses could explain puzzling phylogenies of many DNA informational proteins. *Mol. Microbiol.* **33**, 457–465.

Hall, S.D., and Kolodner, R.D. (1994). Homologous pairing and strand exchange promoted by the *Escherichia coli* RecT protein. *Proc Natl Acad Sci U S A*, **91**, 3205–3209.

Halpern, D., Gruss, A., Claverys, J.P., and El-Karoui, M. (2004). rexAB mutants in Streptococcus pneumoniae. *Microbiology*, **150**, 2409–2414.

Hedayati, M.A., Steffen, S.E., and Bryant, F.R. (2002). Effect of the *Streptococcus pneumoniae* MmsA protein on the RecA protein-promoted three-strand exchange reaction. Implications for the mechanism of transformational recombination. *J Biol Chem*, **277**, 24863–24869.

Hsieh, P., Camerini-Otero, C.S., and Camerini-Otero, R.D. (1992). The synapsis event in the homologous pairing of DNAs: RecA recognizes and pairs less than one helical repeat of DNA. *Proc Natl Acad Sci U S A*, **89**, 6492–6496.

Humbert, O., Prudhomme, M., Hakenbeck, R., Dowson, C.G., and Claverys, J.P. (1995). Homeologous recombination and mismatch repair during transformation in Streptococcus pneumoniae: Saturation of the Hex mismatch repair system. *Proc Natl Acad Sci U S A*, **92**, 9052–9056.

Ivancic-Bace, I., Peharec, P., Moslavac, S., Skrobot, N., Salaj-Smic, E., and Brcic-Kostic, K. (2003). RecFOR function is required for DNA repair and recombination in a RecA loading-deficient recB mutant of *Escherichia coli*. *Genetics*, **163**, 485–494.

Kim, J.I., and Cox, M.M. (2002). The RecA proteins of *Deinococcus radiodurans* and *Escherichia coli* promote DNA strand exchange via inverse pathways. *Proc Natl Acad Sci U S A*, **99**, 7917–7921.

Kodama, K., Kobayashi, T., Niki, H., Hiraga, S., Oshima, T., Mori, H., and Horiuchi, T. (2002). Amplification of hot DNA segments in *Escherichia coli*. *Mol Microbiol*, **45**, 1575–1588.

Kogoma, T., Cadwell, G.W., Barnard, K.G., and Asai, T. (1996). The DNA replication priming protein, PriA, is required for homologous recombination and double-strand break repair. *J Bacteriol*, **178**, 1258–1264.

Kooistra, J., Haijema, B.J., and Venema, G. (1993). The *Bacillus subtilis* addAB genes are fully functional in *Escherichia coli*. *Mol Microbiol*, **7**, 915–923.

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

Kuzminov, A. (1999). Recombinational repair of DNA damage in *Escherichia coli* and bacteriophage lambda. *Microbiol Mol Biol Rev*, **63**, 751–813.

Lloyd, R.G. (1991). Conjugational recombination in resolvase-deficient ruvC mutants of *Escherichia coli* K-12 depends on recG. *J Bacteriol*, **173**, 5414–5418.

Lloyd, R.G., and Low, K.B. (1996). "Homologous recombination." In Neidhart, F.C. (ed.), *Escherichia coli and Salmonella*, Vol. 2. Washington, DC: ASM Press, pp. 2236–2255.

Lloyd, R.G., and Sharples, G.J. (1993). Dissociation of synthetic Holliday junctions by *E. coli* RecG protein. *EMBO J*, **12**, 17–22.

Loughlin, M.F., Barnard, F.M., Jenkins, D., Sharples, G.J., and Jenks, P.J. (2003). *Helicobacter pylori* mutants defective in RuvC Holliday junction resolvase display reduced macrophage survival and spontaneous clearance from the murine gastric mucosa. *Infect Immun*, **71**, 2022–2031.

Lovett, C.M., Jr., Love, P.E., and Yasbin, R.E. (1989). Competence-specific induction of the *Bacillus subtilis* RecA protein analog: Evidence for dual regulation of a recombination protein. *J Bacteriol*, **171**, 2318–2322.

Majewski, J., and Cohan, F.M. (1998). The effect of mismatch repair and heteroduplex formation on sexual isolation in *Bacillus*. *Genetics*, **148**, 13–18.

Marians, K.J. (1999). PriA: At the crossroads of DNA replication and recombination. *Prog Nucleic Acid Res Mol Biol*, **63**, 39–67.

Marians, K.J. (2000). PriA-directed replication fork restart in *Escherichia coli*. *Trends Biochem Sci*, **25**, 185–189.

Marsin, S., McGovern, S., Ehrlich, S.D., Bruand, C., and Polard, P. (2001). Early steps of *Bacillus subtilis* primosome assembly. *J Biol Chem*, **276**, 45818–45825.

Martin, B., Sharples, G.J., Humbert, O., Lloyd, R.G., and Claverys, J.P. (1996). The mmsA locus of *Streptococcus pneumoniae* encodes a RecG-like protein involved in DNA repair and in three-strand recombination. *Mol Microbiol*, **19**, 1035–1045.

McGlynn, P., and Lloyd, R.G. (2000). Modulation of RNA polymerase by (p)ppGpp reveals a RecG-dependent mechanism for replication fork progression. *Cell*, **101**, 35–45.

Michel, B., Flores, M.J., Viguera, E., Grompone, G., Seigneur, M., and Bidnenko, V. (2001). Rescue of arrested replication forks by homologous recombination. *Proc Natl Acad Sci U S A*, **98**, 8181–8188.

Michel, B., Recchia, G.D., Penel-Colin, M., Ehrlich, S.D., and Sherratt, D.J. (2000). Resolution of Holliday junctions by RuvABC prevents dimer formation in rep mutants and UV-irradiated cells. *Mol Microbiol*, **37**, 180–191.

Miller, E.S., Kutter, E., Mosig, G., Arisaka, F., Kunisawa, T., and Ruger, W. (2003). Bacteriophage T4 genome. *Microbiol Mol Biol Rev*, **67**, 86–156.

Morimatsu, K., and Kowalczykowski, S.C. (2003). RecFOR proteins load RecA protein onto gapped DNA to accelerate DNA strand exchange: A universal step of recombinational repair. *Mol Cell*, **11**, 1337–1347.

Muyrers, J.P., Zhang, Y., Buchholz, F., and Stewart, A.F. (2000). RecE/RecT and Redalpha/Redbeta initiate double-stranded break repair by specifically interacting with their respective partners. *Genes Dev*, **14**, 1971–1982.

Myers, R.S., and Stahl, F.W. (1994). Chi and the RecBC D enzyme of *Escherichia coli*. *Annu Rev Genet*, **28**, 49–70.

Narumi, I., Satoh, K., Kikuchi, M., Funayama, T., Yanagisawa, T., Kobayashi, Y., Watanabe, H., and Yamamoto, K. (2001). The LexA protein from *Deinococcus radiodurans* is not involved in RecA induction following gamma irradiation. *J Bacteriol*, **183**, 6951–6956.

Noirot, P., Gupta, R.C., Radding, C.M., and Kolodner, R.D. (2003). Hallmarks of homology recognition by RecA-like recombinases are exhibited by the unrelated *Escherichia coli* RecT protein. *Embo J*, **22**, 324–334.

Noirot, P., and Kolodner, R.D. (1998). DNA strand invasion promoted by *Escherichia coli* RecT protein. *J Biol Chem*, **273**, 12274–12280.

Ochman, H., Lawrence, J.G., and Groisman, E.A. (2000). Lateral gene transfer and the nature of bacterial innovation. *Nature*, **405**, 299–304.

Ogura, M., Yamaguchi, H., Kobayashi, K., Ogasawara, N., Fujita, Y., and Tanaka, T. (2002). Whole-genome analysis of genes regulated by the *Bacillus subtilis* competence transcription factor ComK. *J Bacteriol*, **184**, 2344–2351.

Petes, T.D., and Hill, C.W. (1988). Recombination between repeated genes in microorganisms. *Annu Rev Genet*, **22**, 147–168.

Petit, M.A., Dimpfl, J., Radman, M., and Echols, H. (1991). Control of large chromosomal duplications in *Escherichia coli* by the mismatch repair system. *Genetics*, **129**, 327–332.

Polard, P., Marsin, S., McGovern, S., Velten, M., Wigley, D.B., Ehrlich, S.D., and Bruand, C. (2002). Restart of DNA replication in gram-positive bacteria: Functional characterisation of the *Bacillus subtilis* PriA initiator. *Nucleic Acids Res*, **30**, 1593–1605.

Quiberoni, A., Biswas, I., El Karoui, M., Rezaiki, L., Tailliez, P., and Gruss, A. (2001). In vivo evidence for two active nuclease motifs in the double-strand break repair enzyme RexAB of *Lactococcus lactis*. *J Bacteriol*, **183**, 4071–4078.

Rayssiguier, C., Thaler, D.S., and Radman, M. (1989). The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. *Nature*, **342**, 396–401.

Rinken, R., and Wackernagel, W. (1992). Inhibition of the recBCD-dependent activation of Chi recombinational hot spots in SOS-induced cells of *Escherichia coli*. *J Bacteriol*, **174**, 1172–1178.

Robu, M.E., Inman, R.B., and Cox, M.M. (2001). RecA protein promotes the regression of stalled replication forks in vitro. *Proc Natl Acad Sci U S A*, **98**, 8211–8218.

Roca, A.I., and Cox, M.M. (1997). RecA protein: Structure, function, and role in recombinational DNA repair. *Prog Nucleic Acid Res Mol Biol*, **56**, 129–223.

Roth, J.R., Benson, N., Galitski, T., Haak, K., Lawrence, J., and Miesel, L. (1996). "Rearrangements of the bacterial chromosome: Formation and applications." In Neidhart, F.C. (ed.), *Escherichia coli and Salmonella*, Vol. 2. Washington, DC: ASM Press, pp. 2256–2276.

Saveson, C.J., and Lovett, S.T. (1997). Enhanced deletion formation by aberrant DNA replication in *Escherichia coli*. *Genetics*, **146**, 457–470.

Sawitzke, J.A., and Stahl, F.W. (1997). Roles for lambda Orf and *Escherichia coli* RecO, RecR and RecF in lambda recombination. *Genetics*, **147**, 357–369.

Schapiro, J.M., Libby, S.J., and Fang, F.C. (2003). Inhibition of bacterial DNA replication by zinc mobilization during nitrosative stress. *Proc Natl Acad Sci U S A*, **100**, 8496–8501.

Schmitt, W., Odenbreit, S., Heuermann, D., and Haas, R. (1995). Cloning of the *Helicobacter pylori* recA gene and functional characterization of its product. *Mol Gen Genet*, **248**, 563–572.

Seigneur, M., Bidnenko, V., Ehrlich, S.D., and Michel, B. (1998). RuvAB acts at arrested replication forks. *Cell*, **95**, 419–430.

Seigneur, M., Ehrlich, S.D., and Michel, B. (2000). RuvABC-dependent double-strand breaks in dnaBts mutants require recA. *Mol Microbiol*, **38**, 565–574.

Sharples, G.J. (2001). The X philes: Structure-specific endonucleases that resolve Holliday junctions. *Mol Microbiol*, **39**, 823–834.

Sharples, G.J., Ingleston, S.M., and Lloyd, R.G. (1999). Holliday junction processing in bacteria: Insights from the evolutionary conservation of RuvABC, RecG, and RusA. *J Bacteriol*, **181**, 5543–5550.

Singleton, M.R., Dillingham, M.S., Gaudier, M., Kowalczykowski, S.C., and Wigley, D.B. (2004). Crystal structure of RecBCD enzyme reveals a machine for processing DNA breaks. *Nature*, **432**, 187–193.

Takahashi, N.K., Kusano, K., Yokochi, T., Kitamura, Y., Yoshikura, H., and Kobayashi, I. (1993). Genetic analysis of double-strand break repair in *Escherichia coli*. *J Bacteriol*, **175**, 5176–5185.

Tatusov, R.L., Koonin, E.V., and Lipman, D.J. (1997). A genomic perspective on protein families. *Science*, **278**, 631–637.