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Homologous Recombination—Experimental Systems, Analysis and Significance

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Abstract

Homologous recombination is the most complex of all recombination events that shape genomes and produce material for evolution. Homologous recombination events are exchanges between DNA molecules in the lengthy regions of shared identity, catalyzed by a group of dedicated enzymes. There is a variety of experimental systems in *E. coli* and *Salmonella* to detect homologous recombination events of several different kinds. Genetic analysis of homologous recombination reveals three separate phases of this process: pre-synapsis (the early phase), synapsis (homologous strand exchange) and post-synapsis (the late phase). In *E. coli*, there are at least two independent pathway of the early phase and at least two independent pathways of the late phase. All this complexity is incongruent with the originally ascribed role of homologous recombination as accelerator of genome evolution: there is simply not enough duplication and repetition in enterobacterial genomes for homologous recombination to have a detectable evolutionary role, and therefore not enough selection to maintain such a complexity. At the same time, the mechanisms of homologous recombination are uniquely suited for repair of complex DNA lesions called chromosomal lesions. In fact, the two major classes of chromosomal lesions are recognized and processed by the two individual pathways at the early phase of homologous recombination. It follows, therefore, that homologous recombination events are occasional reflections of the continual recombinational repair, made possible in cases of natural or artificial genome redundancy.

Keywords

Homologous chromosomes; repeats; homologous alignment; strand exchange; recombination intermediate; Holliday junction; crossing-over; conversion; chromosomal lesion; double-strand break; inter-strand crosslink; disintegrated; replication fork; replication fork restart; RecA; RecBCD; RecFOR; RecG; RuvABC

1. Introduction into Homologous Recombination

1.1. Definition of HR and its place among other recombination mechanisms

Changes in DNA are called mutations. Mutations can be changes in one base, in several bases, in many bases. Recombination is also a change in DNA. How is it different from mutation? Generally speaking, recombination is a change in DNA that is not a point

mutation, — for example, change or deletion or addition of several nucleotides. In other words, recombination can be defined as a DNA rearrangement — a change in the order of DNA elements. There are four basic types of enzymatic mechanisms for DNA rearrangements (also see (202) for a similar classification) (Table 1).

This chapter will concentrate on Homologous Recombination (HR). In Biology, *homology* means “fundamental similarity”. Applied to DNA, homology in strict sense means “*sequence identity*”. Thus, “homologous recombination” most frequently represents exchange between two homologous chromosomes — long DNA molecules with essentially the same DNA sequence, containing a few differences.

How is homologous recombination detected? One needs to bring two homologous chromosomes, residing initially in different cells, together in a single cell. In order to be able to detect exchange between the two homologous chromosomes genetically, one also needs a way to separate the two chromosomes (products of HR) into individual cells, to be able to score the phenotypes of individual chromosomes. Finally, to distinguish the two recombinant chromosomes from one another, as well as from the parental chromosomes, one needs scorable traits associated with these chromosomes that results in distinguishing phenotypes at the organismal level. In short, one needs chromosomes carrying phenotype-changing alleles of a pair of genes. Such scorable alleles are called “markers” (Fig. 1A).

From this diagram of the simplest AB x ab cross it is evident why homologous recombination is thought to have a prominent role in evolution: by combining alleles that have arisen in different lineages of the same species, homologous recombination accelerates evolution via generating additional diversity. Under the most prevalent case of stabilizing selection, homologous recombination will enhance elimination of mildly deleterious alleles from the population by combining several of them into organisms that will then have difficulty leaving progeny. However, this role of homologous recombination in evolution of most bacteria in general and *E. coli* in particular is questionable, mostly for two reasons: 1) most bacteria are clonal, with little means of recombinational cross-talk between lineages; 2) the size of typical bacterial populations is such that, with the relatively small bacterial genomes, all combinations of independently-arisen alleles can be generated by mutagenesis alone.

Returning to our definition of homologous recombination, the two chromosomes do not need to be homologous over their entire length for this exchange, as a rather limited region of homology is enough (Fig. 1B). Therefore, homologous recombination is “exchange between two DNA sequences in the region of *shared* homology”. The minimal length of such a shared region of homology fluctuates from one recombination system to another, contributing to confusion about what to consider homologous recombination and what not. The confusion is exacerbated by the fact that short identical sequences are frequently involved in illegitimate recombination events, while the sites by which site-specific recombination events are catalyzed can also be viewed as short regions of homology. Homologous recombination is distinguished from other recombination events not only by the requirement for extended length of homology, but also by the enzymatic systems catalyzing the event. Homologous recombination is catalyzed by specialized and complex

enzymatic machinery, at the core of which lies the still mysterious ability of the homologous recombinase protein (RecA in bacteria, RadA in archaea, Rad51 in eukaryotes) to recognize homology between two DNAs independently of their actual sequence (57). According to this logic, if recombination between 20 bp homologies in *E. coli* is completely prevented in a *recA* mutant, it is considered a homologous recombination event (4). And vice versa, even if the length of homology mediating the exchange is a significant 500 bp, but such a recombination occurs with a good frequency in a *recA* mutant, this RecA-independent recombination is “homology-driven illegitimate recombination” event (reviewed in (31)). To provide some kind of a reference at this point, RecA-dependent recombination, although quite low, is already detectable between 12 base pair-long identical sequences in some experimental systems (290) and becomes the predominant mode of exchange between shared homologies 100 bp or longer in most experimental systems (76, 139, 200, 222, 295).

Finally, an important clarification, illustrated by the trick question: if we flip one of the chromosomes 180° in Fig. 1B, can we still perform the exchange (Fig. 1C)? After all, this would be still an “exchange in the region of homology”. The answer is, of course, “no”, because DNA sequences have polarity (unless they are inverted palindromes), and the regions of homology have to be not only aligned, but also co-oriented to promote recombination (Fig. 1D and E). Therefore, the final definition of homologous recombination is “exchange between two DNA sequences in the region of *aligned* homology, catalyzed by dedicated homology-recognition systems”.

1.2. The principle of HR. The Holliday junction generation and resolution

The exchange in homologous recombination goes through formation of branched DNA molecules, which are then “resolved”. There are several types of branched DNA that can all yield exchange upon resolution; as a convenient example, we will consider the formation of, perhaps, the most complex of these, but whose resolution is the most straightforward. Aligned homologous DNA duplexes (Fig. 2, left) could be imagined to locally melt (Fig. 2, center top) to facilitate formation of the recombination intermediate, in which the strands of the two duplexes are separated and exchanged in alternative pairing to form two hybrid duplexes (Fig. 2, right). Alternatively, the aligned homologous duplexes could be imagined to first form a local quadruplex via the major or minor groove interactions (79, 227, 347) (Fig. 2, center bottom) that then evolves into the intermediate on the right. It should be stressed that neither of the two transient events (Fig. 2, center) was ever detected, — they are shown here to facilitate understanding the nature of the recombination intermediate. This homologous alignment of two DNA duplexes with subsequent strand exchange is the critical step in homologous recombination reaction, catalyzed by dedicated recombinases (the RecA proteins in bacteria). The places in the recombination intermediate (Fig. 2, right) where the two original duplexes end, while the hybrid duplexes begin, are called Holliday junctions, after Robin Holliday who first proposed their existence and explained their importance (121). The formation of Holliday junctions in recombining molecules was subsequently confirmed in electron microscopy studies (30, 259, 260). Specifically, Holliday proposed that DNA junctions are “resolved” by single-strand cleavages into either one or the other *pair* of opposite strands. The two alternative ways to resolve a Holliday junction can be better imagined if we isomerize the junction by rotating two adjacent arms by 180° (260)

(Fig. 3). This operation makes the junction four-fold symmetrical, whereas before it was two-fold symmetrical. Such a fourfold symmetrical junction can be resolved by two-strand cleavage in either one diagonal direction or the other (Fig. 3). It should be stressed that the cleavage affects only two opposite strands at the junction out of the total four. Both ways resolve the junction, freeing the participating duplexes from each other, while the resulting nicks at identical positions in the participating chromosomes are later sealed by DNA ligase (Fig. 3).

Now, where does the exchange of shoulders between participating chromosomes come from? It turns out that cutting the Holliday junction in one direction returns the situation to the parental chromosomes, whereas cutting in the perpendicular direction results in chromosomes that have their shoulders exchanged. Since there are two junctions in our original recombination intermediate (Fig. 2, right), resolution of the second junction also yields either the parental or recombinant arrangement of markers, and the combination of the two becomes a matrix of $2 \times 2 = 4$ possibilities (Fig. 4). Two of these possibilities give parental combinations of shoulders, while the other two give recombinants. Formation of these recombinants is detectable genetically if the parental chromosomes have marked shoulders.

It should be again stressed that Holliday junctions represent only one type of branched DNA molecules, and we considered them mostly for the reason that their resolution is understood best.

1.3. The basic types — HR between homologs, HR between unrelated chromosomes sharing short regions of identity, HR by repeats: deletions, inversions and unequal sister chromatid exchange

We began with defining “recombination” as DNA rearrangements, but eventually spoke of homologous recombination as of something that occurs most often between almost identical chromosomes, which, intuitively, precludes rearrangements. In the above examples, homologous recombination generates chromosomes with new combinations of alleles, originally present on separate chromosomes (Fig. 1A and 4C). Note that the order of genes on the chromosomes is not changed in this process. Exchange between mostly homologous chromosomes is the first basic type of HR and the only one that does not rearrange chromosomes.

Rearrangement is a change in the order of genes on the chromosomes (268). Homologous recombination changes the order of genes on the chromosomes, for example, as a result of the discussed above (Fig. 1D) exchange between mostly unrelated chromosomes in the region of shared identity. This can take the form of “insertion by homology”, if one of the chromosomes was a circular one (Fig. 5A). Recombination can also change the order of genes within the chromosome if the chromosome carries repeats: identical DNA sequences in either direct or inverted orientation. In a reaction that can be viewed as a direct reversal of “insertion by homology”, looping-out at direct repeats mediates formation of a deletion (Fig. 5B). The resulting circles are detectable either genetically (208) or, when the number of tandem repetitions is significant, even physically (282). If the repeats are in the inverted orientation, the product of recombination will be an inversion (Fig. 6A). Finally, unequal

exchange between two identical sister chromosomes carrying direct repeats leads to the simultaneous formation of a duplication and a deletion (Fig. 6B), while such an “alternative” exchange between sister chromatids carrying *inverted* repeats leads to formation of dysfunctional chromosomes and to sure cell death (Fig. 6C) — this is why this latter rearrangement is only detectable in specialized systems (2.3.2.).

The important point to remember is that a chromosome with repeats is susceptible to rearrangements. Another important point to remember is that the frequency of these rearrangements is still very low (10^{-3} - 10^{-4}), so the gross lineage viability is not affected, and one needs a selection to detect these rearrangements. Finally, repeats can recombine without rearranging the chromosome — either by a close double crossover or by gene conversion (Fig. 7) — these recombination events become “visible” if scorable genetic markers are placed *within* the repeats. “Gene conversion” is a homology-guided non-reciprocal transfer of information from one chromosome to another.

1.4. Single crossover versus double crossover in mapping chromosomal genes

HR can be used to map mutations. There are two peculiar properties of HR that allow one to do this. Property #1 is that HR is an infrequent event. In the AB x ab cross, most of the outcomes will be parental chromosomes, and only a few recombinants (Ab and aB) will be obtained. Property #2 is that the probability that an exchange would happen between any two base pairs in DNA is minuscule, but not zero. When there is a significant length of homology between the two markers on the chromosomes, all these tiny probabilities are multiplied thousands and millions of times to result in a detectable recombination. Therefore, the frequency of HR between the two close markers is proportional to the physical distance between the two markers. For a pair of more distant markers, this frequency becomes a complex function of the physical distance. Properties #1 and #2 combined allow one to map markers along the chromosomes, measuring the frequency of their *segregation* from each other (Fig. 8A). For example, if in the cross $a_1b_1c_1 \times a_2b_2c_2$ the frequency of segregation of “a” from “b” is 10%, b from c is 5%, whereas a from c is 13%, and if we know that the chromosomes in this organism are linear, we arrive at the gene order: a - 10% - b - 5% - c (Fig. 8A).

However, this familiar algorithm belongs to the eukaryotic genetics. The eukaryotic life cycle facilitates HR via *syngamy* and *meiosis* (Fig. 8B). Syngamy is a life stage when two entire genome complements are brought together in a single nucleus of a *zygote* in preparation for meiosis. In zygote, every chromosome has its homolog, — an essentially identical chromosome, with a few differences (our “markers”, for example). Zygote can multiply mitotically for some time (in higher multicellular eukaryotes this zygotic clonal multiplication becomes synonymous with the vegetative life stage itself) (Fig. 8B). Meiosis is a special division of a zygote, distinct from mitosis, that regenerates *gametes*, or cells with single chromosomal sets. During meiosis, each pair of homologous chromosomes undergoes from one to several exchanges, so there are no non-recombinant chromosomes in gametes. Finally, all natural eukaryotic chromosomes are *linear*. Any number of exchanges between two homologous linear chromosomes always regenerate monomeric chromosomes.

The nature of viable chromosomal recombinants is quite different in bacteria, because the vast majority of bacteria grow as clones without detectable exchange of genetic information (Fig. 8C), forcing a completely different metric for mapping (Fig. 8D and see below). In the absence of syngamy as a life stage, it is close to impossible to bring in an entire chromosome from another cell. Even if such a chromosome could have been introduced in a bacterial cell, in the absence of the mechanisms to maintain diploidy, such a zygote would be unstable and would immediately segregate the two chromosomes into their own cells. Instead, what is achievable in *E. coli* is to bring an *unstable linear piece* of another chromosome into the cell. However, such an exchange of a whole chromosome with a subchromosomal fragment creates a problem: the chromosome becomes fragmented and, therefore, unstable itself (degradation, inability to complete replication or to finish segregation) (Fig. 9A). To preserve the functionality of the chromosome during recombination with a linear chromosomal piece, two exchanges are required in bacteria (Fig. 9B).

Another common trait of bacterial chromosomes that complicates bacterial genetics is their circularity. Even if there were a way to create a bacterial zygote, — a cell that would contain two whole, genetically marked chromosomes, a single exchange between two circular chromosomes would create a dimer chromosome, which is equally non-functional (Fig. 9C). The solution to this complication again is provided by the double exchange, which preserves the original, monomeric state of the chromosome (Fig. 9D):

Therefore, in bacteria with their mostly circular chromosomes and with the majority of recombinational events involving exchanges with linear subchromosomal fragments, recombinants between chromosomes are always the result of two (or any even number of) exchanges. In these conditions, it is more convenient to look for the simultaneous incorporation of two markers from the subchromosomal fragment, — the so-called *co-inheritance* of the two markers (Fig. 9E). The higher the frequency of co-inheritance in bacteria, the closer the two genes are on the chromosome, *opposite* to what is surmised from recombination frequencies in eukaryotes. For example, if among the progeny that inherited the “a” gene, 60% inherited “b” and 30% “c”, while among those that inherited “b” only 20% inherited “c”, one concludes that the order of genes is c-a-b (Fig. 8D). Parenthetically, since in *E. coli* most of the fine mapping is done with P1 transduction (2.1.2.), co-inheritance also reflects the probability of two loci to be co-packaged in the P1 capsid, which takes ~100 kbp of DNA. The important concept to remember is that, if a genetic cross involves one complete and one incomplete chromosome, or two complete circular chromosomes, it will require two (or any even number of) exchanges to generate viable progeny.

1.5. Viable single crossover and half-crossover events

It should be noted that not all homologous recombination product formation in bacteria requires double crossovers, — only events between two chromosomes do (even if one of them is only a piece of the chromosome, as long as it is linear). Final recombination products can be obtained by single crossover events when recombination happens between closely-spaced repeats on the same chromosome or between the chromosome and a low-

copy-number plasmid. Bacteriophage chromosomes are physically linear during replication and homologous recombination (even though genetically they may be circular), so single crossovers predominate in products of phage-by-phage recombination. Single crossovers are also OK in phage-by-plasmid recombination, when the plasmid is small, while the phage has space to accommodate this extra DNA.

Remarkably, phage-by-phage and phage-by-plasmid crosses reveal yet another type of crossovers, which can be called “half-crossovers” (Fig. 10), representing the “break-copy” principle of homologous recombination (167). In fact, it is currently thought that a significant fraction, perhaps even a majority, of homologous recombination events in the chromosome are of the half-crossover, break-copy type (60, 163), but they are genetically silent because they happen within the same chromosome and reflect recombinational repair of disintegrated replication forks (5.2.).

1.6. Detection of homologous recombination

Specific approaches to detect homologous recombination can be roughly subdivided onto genetic, physical and metabolic ones. Genetic approaches include *selections*, when cells carrying non-recombinant parental markers cannot grow (since this is the overwhelmingly predominant approach, no citations are necessary), and screens — when recombinants look different from non-recombinants, like in papillation or sectoring assays (90, 147). Physical detection looks for 1) the formation of intermediate density species when the two parents have distinct densities (88, 232, 308); 2) change in the overall chromosomal size — for example, multimerization or monomerization of a circular plasmid (87, 145); 3) change in the chromosome configuration — for example, turning circular plasmid into linear concatemeric form (356); 4) change in size of specific fragments (256, 309); 5) stimulation of the experimental chromosome replication — for example, stimulation of plasmid replication by an infecting phage with shared homology (155, 220). Polymerase chain reaction allows one to detect even the elusive recombination intermediates, especially during synchronized reactions in eukaryotes (314); however, due to the extremely fast rate of recombination in *E. coli* and *Salmonella*, this method is less applicable in these organisms. Finally, metabolic detection relies on transcription of the recombinant DNA to yield detectable product of the restored gene (24, 193).

2. Specific recombination systems

Three basic formats of recombination reactions in relation to the topology of the participating substrates are possible: 1) circular-by-circular; 2) circular-by-linear; 3) linear-by-linear (Fig. 11). Recombination in *E. coli* can be detected between two chromosomal DNA sequences, or between the chromosome and an extrachromosomal genetic element (phage, plasmid), or between two extrachromosomal elements. When the chromosome is involved, the cell is a merozygote (= partial zygote) by definition, but when both recombining DNAs are extrachromosomal, the *E. coli* cell is most of the time a complete zygote for those DNAs, even if a transient one in crosses involving phages.

2.1. Chromosome-based unstable merozygotes

When a linear piece of a different chromosome comes to a recipient cell via conjugation with an Hfr strain, or as a result of transduction or transformation, the resulting merozygotes are unstable because the introduced piece does not have its ends protected and will be eventually degraded by the cellular exonucleases, if not incorporated into the resident chromosome. The entering linear piece may even have a replication origin — but the ensuing replication, revealed by production of multiple recombinants from a single exconjugant cell (196, 251, 349), does not matter as long as the piece stays linear, because it is unstable. The topological format of this recombination is circular-by-linear, and the formation of viable products always requires double crossovers.

2.1.1. Conjugative recombination—Bacterial cells are frequently infected by big plasmids that spread to uninfected cells of related bacteria by building conjugation bridges between two cells, spooling through the bridge one DNA strand starting from a specific “transfer” origin and replicating it in the recipient cell into a duplex (101, 335). In *E. coli*, such a plasmid is called “F”, from “fertility factor” (127, 141, 171, 343). When the F-plasmid is integrated into the host chromosome (usually via homologous recombination (44, 62) at IS elements (70), but sometimes as a result of transposition of these elements itself (327)), it still tries to conjugate into a susceptible bacterium, now carrying the whole host chromosome with it (133). The strain that has F-plasmid in the chromosome is called Hfr (for “high frequency of recombination”), or “male”, or “donor” strain, while the susceptible strain without F-plasmid is called the F-, or “female”, or “recipient” strain (64). The mating bridge between the male and female strains is built to support transfer of DNA much longer than the 100 kbp F-plasmid; however the DNA transfer itself is quite vulnerable to unknown chromosomal factors (348), — as a result, even under optimal conditions, the probabilities of transferring one-quarter, one-half and the entire chromosome can be calculated as only 0.2, 0.039 and 0.0015 (302). Transfer of an incomplete chromosome, a sub-chromosomal fragment, turns the recipient cell into a merozygote, in which the circular resident chromosome can recombine with the sometimes quite long linear piece of the Hfr (“donor”) chromosome. In 80% of the cases, such recombination leads to incorporation of the bulk of the linear DNA as a single “chunk” into the recipient chromosome; in the rest 20%, shorter segments of the donor subchromosomal piece are incorporated into the donor chromosome independently of each other (302).

The frequency of such recombination after conjugation depends on several factors, the major ones being: 1) the frequency of conjugational transfer itself, which may vary from one donor-recipient pair to another; 2) the DNA replication proficiency of both the donor and the recipient strains; 3) the recombination proficiency of the recipient; 4) the level of DNA damage in the donor strain. For example, if the donor cells are treated with DNA-damaging agents before transfer, much shorter pieces of their chromosome are incorporated into the recipient genome; if the recipient cells are treated instead, the frequency of recombinants (relative to the survivors) is increased (342, 350).

Sometimes after integration into the donor chromosome, F-plasmid breaks free, picking a piece of the chromosomal DNA with it; such F-plasmids carrying segments of the host

chromosome are called F' (201). When an F' conjugates into a recipient cell, it is stably maintained there, making the cell diploid for the chromosomal region the plasmid carries. In these stable merodiploids, two types of recombinants are detected: F'-plasmid integration into the chromosome by homology and the exchange between chromosomal and F'-plasmid-carrying markers, frequently a non-reciprocal one (201) (and also see 2.2.1.). Even if no recombination is detected, F' transfer itself is useful for normalization of the conjugation- and DNA replication-proficiency of this donor/recipient pair (190).

2.1.2. Generalized transduction—Bacteriophages infect bacterial cell and utilize its material to replicate and package their genomes, then lyse the emptied bacterium to infect new cells (32). Bacteriophages are specifically designed to either shred the host DNA to utilize its nucleotides or, if the host chromosome is not degraded, at least to avoid packaging pieces of it, but many of them do it at low frequency anyway (218). When such a “phage” carrying only a piece of bacterial chromosome infects a cell of the same or closely-related bacterial species, the injected sub-chromosomal piece can incorporate via homologous recombination into the resident chromosome, bringing in new genes or different alleles of resident genes. Such a transfer of genetic information from one bacterial cell to another with the help of a bacteriophage is called “generalized transduction” (218). Generalized transduction can be observed with any virulent or lytically-developing temperate bacteriophage (although with most of them its detection requires either special phage mutants or conditions) and transfers any chromosomal piece with a low and more-or-less equal probability.

Certain phages can be mutated to corrupt the specificity of their packaging machinery — this makes them better “transducing phages” (312). The widely-used transducing phage in *E. coli* is a derivative of P1 (capsid takes about 100 kbp worth of DNA), for *Salmonella* it is a derivative of P22 (capsid takes about 44 kbp of DNA) (218). While in typical transducing phages the fraction of transducing particles is less than 1% and never more than 5%, mutant phages are known in which up to 50% of the virions can be filled with the random pieces of host DNA (289, 332).

Although the injected piece in the case of P1 transduction is full 100 kbp, the median size of the integrated pieces is only 20% of the total size, ~20 kbp (218). Still, markers separated by 60 kbp co-integrate with 0.1x frequency of the integration of a single marker. For a much smaller P22 of *Salmonella*, the numbers relating integration frequencies with the length of integrated pieces are surprisingly similar (218). Integration is again in “one chunk” (78, 236). Generalized transduction is by far the most common and easiest way to transfer mutations between strains in *E. coli* and *Salmonella*. Moreover, it can be also used to select for (or to generate?) duplications in the recipient chromosome; counterintuitively, the duplicated chromosomal segment in this case can be much longer than the transduced piece itself (6, 118). In experienced hands, transduction becomes a kind of genomic Lego, as complex chromosomal rearrangements can be assembled in several transducing steps from suitable donors (268).

2.1.3. Phage-by-chromosome events and specialized transduction—

“Specialized transduction” is a phenomenon observed with a sub-class of temperate phages

that, as lysogens, reside in the host chromosome. As a result of improper excision events, these phages occasionally spawn hybrid variants carrying stretches of contiguous chromosomal regions (337). Lysogenization with such a hybrid phage via the regular site-specific recombination at the *attB* site generates a bacterium with a duplication of the captured region, and the majority of the specialized transduction events are of this type, which has nothing to do with homologous recombination. However, lysogenic induction of phage Lambda, for example, also generates defective phage particles, called Lambda *doc*, that result from attempted packaging of the Lambda copy that is still in the chromosome, and therefore approximately half of DNA in these particles is the neighboring bacterial DNA, either to the right or to the left of the prophage (337). Recombination of such a hybrid phage with the chromosome is by chromosomal homology (338) and resembles generalized transduction, only it is specific to the regions contiguous with the prophage — hence the term “specialized transduction”.

The read-out in transduction is always formation of a recombinant bacterium, so the fate of the phage DNA, originally attached to the transduced chromosomal piece, is unknown. However, the situation can be turned around, when the chromosomal homology is constructed into the phage genome, and the recombination is followed by detecting recombinant phages that “picked up” markers from the chromosome, in either heteroimmune or homoimmune crosses (25, 177). Probably one of the more dramatic examples of such recombination is the formation of the Lambda-reverse phage. A mutant lambda, deficient in its own phage recombination functions, cannot grow well on the wild type *E. coli*. This growth defect in wild type *E. coli* is suppressed when the mutant lambda picks up the *recET* genes of the defective *Rac* prophage from the host chromosome (94, 97, 136). Although *Rac* is mostly heterologous with lambda, short regions of shared homology apparently provide enough information to make a successful recombinant, as proposed for other phage-by-phage crosses (38).

2.1.4. Transformation—Although a few bacteria are naturally transformable with exogenous DNA from the environment (195, 313), most bacteria do not take in exogenous DNA unless artificially forced to become “competent”, and *E. coli* is not an exception. *E. coli* cells can be made competent (= transformable, capable of taking in DNA) by treatment with divalent cations (especially Ca^{2+}) (105, 106, 214). Calcium phosphate has a low solubility, and DNA, being a polyphosphate in nature, “precipitates” on the cell surface LPS in the presence of Ca^{2+} (252), which facilitates subsequent DNA internalization, via still unknown mechanisms, triggered by a transient heat-shock. However, the best way to introduce DNA into *E. coli* remains electroporation in the extremely high and extremely brief electric fields (106, 237), when DNA molecules become projectiles and forcefully penetrate into bacterial cells (138).

Once the problem of DNA uptake is overcome in *E. coli*, a linear DNA that wants to incorporate into *E. coli* chromosome by homology faces another problem: degradation by the RecBCD exonuclease (19, 330). This makes recombination after exogenous DNA introduction by either chemical or physical means not a very efficient process in wild type *E. coli* (although still the last chance, if P1 transduction cannot be done) (80), but the situation can be significantly improved in *recBC sbcBC* or *recD* mutants of *E. coli*, in which

alternative recombination pathways operate (52, 119, 275, 331), or if the host recombination enzymes are replaced altogether with those of lambdoid bacteriophages (244, 355, 358). In fact, the chromosomal incorporation of homologous DNA by phage recombination functions is so efficient, that PCR products (68) and even oligonucleotides (53, 359) are now routinely used to design new chromosomal DNA sequences in vivo (287, 321) (see Eco-Sal III Module 7.2.9. λ Recombination and Recombineering).

2.2. Chromosome-based stable merozygotes

Merozygotes are stable when two (or more) homologous sequences can stably propagate together in the same cell. This situation is realized when there are plasmids in the cell carrying pieces of the chromosomal DNA, or there are repeats on the same chromosome. The format of this recombination is circular-by-circular, although mechanistically it is likely to be a mixture of circular-by-circular and circular-by-linear events (ends of the double-strand breaks). Some of the resulting viable recombination products can be formed by single crossovers.

2.2.1. Plasmid-by-chromosome events—When plasmids, — autonomously-replicating and much smaller than the host chromosome DNA molecules, — carry segments of the chromosomal DNA, recombination between the plasmid and the chromosome can insert the former into the latter (Fig. 5A) or can exchange plasmid markers into the chromosome and vice versa, if they carry distinctive markers. The frequency of these events is 10^{-4} , so their detection requires extensive screening (173), unless damage to plasmid DNA is used to stimulate recombination (242). Conveniently, two types of powerful selection are available to reveal the plasmid integration events.

One way to select for plasmid integration is to use *dnaA*(Ts) mutants at the non-permissive temperature. DnaA is the origin-recognition and replication-initiation protein in *E. coli* and *Salmonella*. In the absence of initiation from *oriC*, origins of the integrated plasmids provide an alternative route for initiation of the chromosomal replication. Both high copy number plasmids (215, 351) and low copy number plasmids can integratively suppress this *dnaA*(Ts) initiation defect; however, fully-functional origins of multicopy plasmids are tolerated in the chromosome only in the presence of the free plasmid copies of the same origin (351). Apparently, in the absence of multiple copies of free plasmid to titrate the diffusible plasmid initiator, multiple initiations from the plasmid origin within the chromosome kill the cell. In contrast, chromosomal insertions of plasmids with the copy number 1-2 per chromosome, like F or R, are well-tolerated (67) and fully suppress the chromosomal replication defect of the host without preconditions (217, 247).

The other way to select for plasmid integration into the chromosome by homology is to move the cells to conditions non-permissive for plasmid replication, while keeping selection for plasmid genes (93, 104). For example, since ColE1-type plasmid replicons depend on DNA polymerase I for initiation of their replication, shifting *polA*(Ts) mutants to the non-permissive temperature while maintaining selection for a plasmid gene (usually antibiotic-resistance) forces the plasmid to seek integration into the chromosome, which can be facilitated by prior cloning of a chromosomal sequence onto the plasmid (103). Returning

the cells to conditions permissive for plasmid replication forces the chromosome to shed the plasmid, because even an integrated replicon with 6-10 copies per chromosome significantly slows cells down (104). This way, *polA* mutants can be used to pick up the chromosomal markers onto plasmids (276). A more efficient alternative is provided by P1 transduction of a plasmid that carries homology to the host chromosome with differences in alleles: the transduced plasmid is enriched for the chromosomal allele, apparently via transient plasmid integration into the chromosome (176).

As already mentioned above (2.1.1.), the F-plasmid can integrate into the chromosome (via transposition, or more frequently, via homologous recombination with pre-existing IS elements (70)), forming an Hfr strain, but then can sometimes leave the chromosome, carrying the piece of the latter, to form “primary” F’. Strains with “primary” F’ frequently cannot lose the enlarged episome, because the still haploid chromosomal region, now on the plasmid, carries essential genes. Once transferred to a wild-type F– strain, F’ becomes “secondary”, meaning that it confers diploidy on the chromosomal region it carries (201). A secondary F’ can either exchange markers with the chromosome (if there are distinct markers to begin with) (115, 133, 345) or it can integrate into the chromosome. Integration of secondary F’s by homology into the chromosome is the reason F’-strains mobilize the chromosome during conjugation with high frequency (254, 288, 341).

2.2.2. Repeat-mediated recombination—When the chromosome carries repeats, — stretches of DNA that have homologies in either direct or inverted orientation someplace else on the same chromosome, *single* crossover between these repeats in non-replicating chromosomes can lead to two classes of chromosomal rearrangements: 1) deletion between direct repeats (Fig. 5B); 2) inversion between inverted repeats (Fig. 6A). In the replicating chromosomes, two more rearrangements are possible: 3) direct repeats can cause unequal sister chromatid exchange (Fig. 6B), while inverted repeats can cause dimerization with inversion (Fig. 6C and 11), the latter most likely lethal in the chromosome, because it has never been detected (10) (1.3., but see 2.3.2. below for viable products of this recombination). In contrast, *double* crossovers between repeats will only exchange markers within the repeats themselves, without rearranging the chromosome (Fig. 7). Finally, homozygotization of repeats (when genetic differences between the two repeats are erased in favor of one of the repeats) can also happen and is referred to as “gene conversion” (280) (Fig. 7).

Although bacterial genomes are generally considered non-repetitious (29), they do carry around 1% of their DNA in repeats, which, in the genomes of *E. coli* and its relatives, are of four kinds: 1) ribosomal RNA (rRNA) operons — both *E. coli* and *Salmonella* have seven of these 6 kbp-long repeats (26, 224); 2) the mysterious *Rhs* elements (up to eight in some strains, belonging to up to five different lineages) that share a 3.7 kb-long core (333); 3) insertion sequences, 0.8-2.0 kb-long, whose actual copy number vary from strain to strain of *E. coli* or *Salmonella*, but for any given element is usually below 10 (70, 327); 4) small extragenic repeats (BIMes/REPs, IRUs, box C sequences, RSA sequences) (11), that are too short and imperfect to be efficient recombination mediators, but whose influence is still felt because of their sheer numbers that for some of them run into hundreds.

Recombination between rRNA operons creating duplications of chromosomal segments is argued to be quite frequent, on the order of 10^{-4} to 10^{-3} in the population, but too deleterious to change the chromosomal structure in *E. coli* or *Salmonella* in the absence of a strong selection for the resulting duplication (6, 7, 116). Deletions between rRNA operons would be too deleterious for survival, but inversions, especially those that do not significantly change the position of the replication origin, are well-tolerated (117). It is surprising, therefore, that the natural *E. coli* and *Salmonella* isolates keep the same gene order in the origin-proximal chromosomal half, delineated by the rRNA operons (116), especially so since the high degree of homology between these ancient duplications (“concerted evolution”) suggests regular homologous recombination events in the form of gene conversion (175). Interestingly, in contrast to this “set-in stone” chromosomal frame of most *E. coli* strains and *Salmonella* serovars, *Salmonella typhi* chromosomes are notoriously rearranged by inversions and transpositions between rRNA operons (183, 278).

Recombination between the related *Rhs* elements can be as frequent as the one between rRNA operons (179). Recombination between insertion sequences can invert significant chromosomal segments (108) and whole chromosomal arms (197), integrate F-plasmid to generate Hfr strains and produce F’ plasmids, carrying chromosomal pieces (327) (discussed in (70); see also 2.2.1.). Recombination between REP elements is also documented (143, 299).

Deletions between closely-spaced direct repeats that do not remove essential genes are detected on the chromosome (35, 199), but recombination between widely-spaced direct repeats can go only through double-crossovers or gene conversion, to avoid deletion of essential genes. The same is true for the majority of inversions that do not include either the replication origin or the terminus: single crossovers are only allowed if the inverted repeats are not too far away from each other (291), while both formation and tolerance of longer inversions are governed by several restrictions (33, 291). One classic example of exclusively double crossover / gene conversion events mediated by widely-spaced inverted repeats is the Konrad system for detection of hyper-rec mutants (147, 360, 361), in which neither origin, nor terminus is included between repeats. On the other hand, recombination between the two 99%-homologous genes (*tufA* and *tufB*), coding for EF-Tu translation elongation factor and present in the chromosomes of both *E. coli* and *Salmonella*, can go by either single crossover or by double crossover / gene conversion, because the two repeats are positioned almost symmetrically around the replication origin in the inverted orientation. In *Salmonella*, recombination between genetically-marked *tufA* and *tufB* genes goes by both single crossover or double crossover / gene conversion (1, 126), and the non-reciprocal events predominate in the latter case, suggesting gene conversion, rather than actual double crossovers (9).

2.3. Extra-chromosomal systems

These offer not only an added flexibility, since the smaller size of the extrachromosomal substrates facilitates their physical handling and reconstruction, but also one more substrate configuration: linear-by-linear, in phage crosses. Moreover, the substrate input can be tightly controlled in this case, while the product output tends to be orders of magnitude higher than

in other systems, reaching absolute numbers comparable with those of initial substrates. Because of this unusual efficiency, the extra-chromosomal systems are favored by those operating genetically at the lower limits of homologous recombination mechanisms (“extreme recombination”), as well as by those studying the in vivo molecular mechanisms with physical techniques (“in vivo biochemistry”).

2.3.1. Phage-by-phage recombination—Recombination is both robust (due to several recombining phage genomes per each cell) and synchronous (due to synchronous and rapid injection) among phages, making this the most powerful recombination format known, equally suitable for investigations with genetic, biochemical or physical techniques (163, 240, 319). Phage recombination is generally robust because phage DNA replication generates a lot of waste, so phages like lambda use recombination in part to recycle this waste, maximizing the yield of their packageable DNA (163). This ensures close to one exchange per progeny lambda chromosomes, which is perfect for genetic analysis. Other phages, like T4, depend on homologous recombination to prime their late DNA replication and will stop their development if homologous recombination is blocked by mutation (241). These phages enjoy several exchanges per every packaged phage genome, which makes their recombination almost “too hot” for genetic analysis at the genome scale, yet perfect for physical detection of in vivo recombination products (156) or for fine genetic analysis at the scale of a single gene (18, 301). And, of course, the frugal yet sophisticated (rather than the primitive phage-like) biochemistry of T4 homologous recombination yields a bonanza of insights about the enzymology of homologous strand exchange, not only in bacteria, but also in eukaryotes (14, 153).

Small bacteriophages tend to replicate prudently and therefore recombine much less than their bigger cousins (274), but their recombination can be induced in the presence of recombination systems of other phages (243) or by DNA damage (17). In fact, the format of the phage cross (or a simple infection), in which phages are pretreated with DNA damaging agents before infection, is a perfect way to circumvent the uncertainty principle of the DNA repair research. The uncertainty derives from two contradictory requirements (as in quantum mechanics, where the first uncertainty principle was ever formulated). To understand the DNA repair mechanisms, ideally one would like to know how the cell repairs a few “pure” DNA lesions. However, in order to reliably *detect* DNA lesions and their subsequent repair in vivo with physical techniques, one needs to introduce *massive* DNA damage. Massive DNA damaging treatments perturb the normal cell metabolism because of the unavoidable damage to other cellular components, like RNA, proteins and membranes, and because of the cellular responses to massive DNA damage that can change the enzymology of repair itself (like SOS response in *E. coli* (see Eco-Sal III Module 5.4.3. “The SOS Regulatory Network”, and Module 7.2.8. “The SOS Response”)). Using phages, it becomes possible, without treating cell with DNA-damaging agents, to task the cell with a considerable (= easily detectable) DNA repair / recombination effort on phage DNA that was pre-treated before infection.

Use of temperature-sensitive phage mutants, or suppressor-sensitive phage mutants in conjunction with suppressing and non-suppressing host bacteria, makes crosses between two or more phages easy to set up (84). Alternatively, a single phage parent carrying repeats can

be used, scoring deletions or triplications with direct repeats (16, 112) or inversions with inverted repeats (82, 140). Phage systems can be also used to study how inhibitors of various aspects of the host cell metabolism influence recombination (151, 325). Finally, recombination between related phages can be studied (15). Another unique advantage of the phage-by-phage crosses is the fact that recombination acts are separated from subsequent analysis of recombinants via “rescuing” the products of recombination through packaging. The packaged products are later analyzed in host cells that could have either the same or different genotypes, to facilitate scoring of various phage markers. Such a separation of the recombination act from subsequent analysis allows one, for example, to completely block DNA replication during the cross and still retrieve a few recombinant particles, revealing the important role of replication in homologous recombination (309). Moreover, since the final recombination product is packaged linear, relieving the critical requirement for regeneration of the circular chromosome, not only linear-by-circular, but also linear-by-linear crosses are productive.

One of the early favorites in the study of the general principles of homologous recombination (catalyzed by phage-encoded enzymes), the phage cross can also be used to study *E. coli*'s homologous recombination, by employing recombination-deficient phages (261). Phages like T4 depend on their own hyper-efficient homologous recombination for phage DNA replication (241) — therefore, they cannot be used to study host cells' recombination. On the other hand, phage lambda can in principle replicate without recombination (83), while recombination-deficient lambdas recombine happily using the host homologous recombination pathways (307). Thus, the repressed phage lambda chromosome is a perfect substrate to study the relations between such complex phenomena as double-strand break repair by host recombination system and DNA replication (167), or even phage own recombination, when the recombination functions are expressed from plasmids (257, 309). In fact, were it not for phage crosses catalyzed by the host enzymes, such an important aspect of the regulation of the bacterial homologous recombination as turning off the RecBCD-catalyzed DNA degradation at Chi-sites (reviewed in (245)), would have been discovered only 20 years later (66).

2.3.2. Plasmid-by-plasmid recombination—Although evidence of recombination was periodically surfacing in natural-replicon-based plasmids, like a deletion between IS2 direct repeats within F' (71) or recombination intermediates in ColE1 (259, 260), this strictly circular-by-circular format of homologous recombination between or within small extrachromosomal circles became really popular with the advent of genetic engineering, allowing one to design experimental systems targeting detection of specific events of homologous recombination at the very limits of detection (31, 45). In fact, in plasmids based on engineered replicons devoid of the multimer-resolution systems, the most graphic and easily-obtained physical evidence of homologous recombination is plasmid multimerization (13, 87, 356) and demultimerization (145).

Two general classes of substrates for genetic detection of plasmid-by-plasmid recombination are employed: one class consists of two compatible (stably maintained together in the same strain) plasmids carrying regions of mutual homology (135, 169, 170, 200) (this corresponds to multimer formation in the physical assays), while the other class is a single plasmid with

repeats of various lengths, either in direct (21, 72, 199, 222) or inverted (20, 212, 352) orientation (corresponding to demultimerization in physical assays). While the first class has to recombine *intermolecularly*, the second class can recombine either inter- or *intramolecularly*, with both types of events detectable (199, 352). Sometimes the repeated sequences carry heterologies, the setup that allows one to detect gene conversion-type events (74, 86). Finally, since plasmids are relatively small, their introduction into the host cells by transformation is rather efficient compared with the chromosomal DNA, which allows one to transform bacterial cells with linearized plasmids or even their pieces (both would be unable to replicate) to explore the mechanisms of double-strand break-stimulated recombination by isolating replication-competent circular products of the forced reassembly (69, 142).

Although plasmid-by-plasmid recombination sounds like a little (=inadequate) brother to the chromosomal recombination, miniaturization of the substrates gives plasmid recombination at least two advantages over its big brother. First, physical analysis of the overall configuration of the recombination products becomes possible, providing interesting insights about the structure of the plasmid dimers (21, 72, 199, 222). For example, in addition to the expected reciprocal 1+3 dimers, direct repeats also mediate formation of the mysterious non-reciprocal 1+2 dimers (72, 222), which are also observed in mitochondrial DNA rearrangements in higher eukaryotes (162). Another interesting example is formation of the outlandish-looking head-to-head dimers, mediated by inverted repeats (Fig. 12) (20, 206, 212). Such analysis is impossible with the chromosome, in which tandem dimers, even if they form, are quickly resolved into monomers by the XerCD/*dif* system in preparation for segregation and cell division (296), while inverted dimers (Fig. 12) are simply inviable (10).

And second, analysis of the products of plasmid recombination is not restricted to the cell in which they originally formed: plasmid DNA can be isolated and introduced for analysis into a different strain (usually, a recombination-deficient one to suppress further recombination) (13, 74, 135, 260). This trick is especially useful with multicopy plasmids, in which, due to their enormous copy number, selection for the rare (recombinant) variants is inefficient (223). This property of crosses in the high-copy number plasmids should be kept in mind when interpreting obligatory dimerization of the recombinant plasmids (162), since dimerization accelerates replication of plasmids, regulated by a common diffusible inhibitor (315) and, therefore, enhances the selection.

2.3.3. Plasmid-by-phage events—Plasmids can form as by-products of aborted phage infection (like lambda-dv (219)) and can also recombine with the infecting phages if they carry segments of mutual homology. Genetically, if the phages are the final read-out, two outcomes are possible: either plasmid integration by a single cross-over (the plasmid is small, while the phage chromosome has space) (139, 221, 295, 320), or the phage picking up a marker from the plasmid as a result of a double crossover (301, 306, 320) (especially if the plasmid is too big to splice into the phage). Double crossovers are the only outcome detectable if the restoration of the plasmid is the read-out, as in the phage-mediated transduction of plasmids, carrying segments of the phage genome (154, 248). Physical analysis of the products of plasmid-by-phage recombination is possible if the size of the products is expected to be different from the size of the substrates: the small size of both

recombining molecules facilitates detection of differences using standard DNA separation techniques like gel electrophoresis (256). Alternatively, stimulation of plasmid replication by an infecting phage, if they share homology, can be observed (155, 220).

Due to the robust and efficient nature of crosses involving bacteriophages (see 2.3.1.), even physical detection of otherwise elusive *recombination intermediates* becomes possible. Importantly, some of these intermediates are dead-end products, undetectable genetically, and their physical detection offers recombination studies the depth of resolution which is only contemplated in other DNA metabolism studies, like mutagenesis (see Eco-Sal III Module 7.2.1. Mutagenesis). For example, recombination with the phage stimulates rolling-circle replication in a plasmid, which is detectable physically, but cannot yield stable final products, detectable genetically. The plasmid-by-phage setup was the first to demonstrate recombination-dependent replication, an indication that homologous recombination generates functional replication forks (155, 220). Physical demonstration of this event in the chromosome would be close to impossible due to much lower frequency of these events there. In fact, plasmid-by-phage recombination is so robust and efficient that it was successfully used to study the minimal sequence length requirements of homologous recombination in *E. coli*, which turned out to be around 30 nucleotides (139, 295, 301, 336).

3. Genetic analysis of HR reveals distinct pathways

Homologous recombination is a complex phenomenon with mechanisms that are still not completely understood, especially in the areas of generation of recombination substrates and also where recombination connects with DNA replication. Genetic characterization of the distinct pathways of homologous recombination in *E. coli* was facilitated by the availability of diverse experimental systems (see 2.) and by the strong physiological phenotypes of recombination-deficient mutants. Recombination-deficient mutants are sensitive to DNA damage, which not only offers a convenient read-out, but also indicates that the main physiological function of homologous recombination is repair of chromosomal damage ((163); also see below and Eco-Sal III Module 7.2.7. Recombinational Repair — Enzymes and Pathways). However, DNA damage sensitivity of a mutant does not automatically qualify it as a recombinational repair one, — it is more likely to be a *bona fide* DNA repair mutant (see Eco-Sal III Module 7.2.4. DNA Damage Reversal and Excision Repair), so homologous recombination tests (see Section 2. above) should be used to verify that the mutant indeed affects homologous recombination. And even in the case of a DNA damage-sensitive and homologous recombination-defective mutant, care should be taken to separate true recombination functions from those of DNA replication and chromosome condensation and segregation. Indeed, recombination operates on DNA organized in nucleoids and creates substrates for subsequent chromosomal replication, — therefore detection of recombinant chromosomes usually assumes their successful replication, condensation and segregation. Likewise, survival of DNA damage critically depends on restoration of DNA replication, likely in the context of the nucleoid. Thus, partial defects in DNA synthesis elongation, like *dnaE* (27), or restart, like *priA* insertion mutants (144, 279), or in the nucleoid-associated proteins, like HU (75, 137, 174), may behave like homologous recombination mutants without being those as such; these chromosomal replication and organization functions are discussed elsewhere in Eco-Sal III (128, 157).

3.1. Isolation of mutants in homologous recombination

There are very few circumstances under which homologous recombination becomes poisonous to the cell, and these already involve some other defects in homologous recombination, making selection or even enrichment for recombination-deficient mutants in otherwise wild type *E. coli* generally unavailable, and leaving only the possibility of brute-force screens. Some mutants in homologous recombination (mnemonic “*rec*”), notably *recA* (40) and *recJ* (198), were isolated by screening for defective recombination after conjugation (2.1.1.) on replica plates (reviewed in (36, 39)). There were also other mutants defective in conjugational recombination, but since they also had defects in other DNA transactions, they were not specific for homologous recombination. However, most of the early mutants were isolated differently, by screening for the X-ray or gamma-ray-sensitivity and then also checking their recombination-proficiency; mutants in *recA*, *recB*, *recC*, *ruv(ABC)* and *recG* were isolated this way (81, 125, 187, 250, 328). Since the major chromosomal challenge after X-ray or gamma-ray radiation is repair of double-strand DNA breaks, while conjugational recombination proficiency also depends on double-strand break repair, it is not surprising that all the early mutants were defective or deficient in this repair.

Once a *rec* mutant was isolated in otherwise wild type cells, the next step was to attempt isolation of its extragenic suppressors that would restore recombination-proficiency. In this way, it was found that the *recA* mutants could not be suppressed indirectly, but *recB*, *recC* and *ruv* mutants were easily suppressed, usually by activation of analogous functions residing on defective prophages. Specifically, the *sbcA* suppressors returned recombination-proficiency after conjugation to both *recB* and *recC* mutants by activating the RecE exonuclease and the RecT strand annealing protein (12, 95) of the Rac prophage (346), whereas the *rus* suppressors did the same to the *ruv* mutants (294) by activating an alternative resolvase RusA of the DLP12 prophage (211). Curiously, the *recG* defect in recombination and DNA damage sensitivity was suppressed by inactivation of the non-essential helicase activity of the otherwise nearly-essential primosome-loading protein PriA (3), suggesting that RecG is not a true recombination function, but instead controls some potentially poisoning pathways associated with DNA repair.

Once the restoration of recombination proficiency was achieved, a new search for recombination-deficient mutants, now in a double mutant background, became possible. These mutants would now reveal the functions important for the alternative pathway that was suppressed in the wild type cells. Mutations inactivating homologous recombination in the *recB sbcA* or *recC sbcA* background include the expected *recA* (95), *recE* (42, 95) and *recT* (42), but also *recF* (95), *recJ* (198), *recO* (146), *recR* (209) and *ruvC* (190). The only mutation that was found to inactivate homologous recombination in the *ruv rus* background (besides the expected *recA* and *recBC* defects) was *recG* (211, 213). Symmetrically, the *ruv* defect returned DNA damage-sensitivity to the *recG priA*(helicase-minus) double mutants (134).

Quite a different logic of extragenic suppression is exemplified by the *recBC sbcBC* recombination-proficient mutant combination. In this case, suppression is conferred by two unlinked mutations, *sbcB* (317) and *sbcC* (188) (or *sbcD* (92)), and the mechanism of it can

be considered opposite to the *recBC sbcA* suppression: instead of activating an analogous function to compensate for the *recBC* defect, the *sbcBC* suppressors dramatically modify the metabolism of linear DNA in *E. coli* cells. The modification includes inactivation of the nuclease domain of ExoI, the ssDNA-specific exonuclease and the product of *sbcB/xonA* gene, turning it into a 3'-ssDNA-end-protecting protein (322), as well as complete inactivation of the SbcCD nuclease that attacks DNA hairpins and can also trim ssDNA overhangs of a duplex via its ss-endo activity (49, 50). However, the recombination-deficient mutants, isolated in the recombination-proficient *recBC sbcBC* genetic background, are the same as for the *recBC sbcA* background, *sans recE* and *recT*, the two λ prophage recombination functions that are not activated in the *sbcBC* mutants in the first place. Specifically, *recA* (123), *recF* (123), *recJ* (123, 198), *recO* (146), *recR* (209) and all three *ruv* genes (186, 213) are needed for high conjugational recombination in the *recBC sbcBC* background. In addition, the *recG* (187), *recN* (190), *recQ* (231, 246) and *uvrD*, the latter one either alone (123) or together with *helD* (231), genes also participate, emphasizing the differences between the two pathways. The list of *rec*- mutants in the *recBC sbcBC* background includes only those whose specific defect was in homologous recombination and in DNA damage repair. Conjugational-recombination-deficiency indices for all the recombination mutants in wild type, *recBC sbcA* and *recBC sbcBC* backgrounds are shown in Table 2.

Isolation of the secondary recombination-deficient mutations in the suppressed *rec* mutants naturally lead to the idea of several recombinational pathways in *E. coli*, the major one (RecBC) and two minor ones (RecE and RecF), the latter two normally suppressed in the wild type cells (36, 37, 41). It is worth noting in retrospect that, although the original “recombination system” with three pathways turned out to be too “conjugation-centric” to be universal, the general idea of separate recombinational pathways was fully validated (see 3.2. and 3.3.). Specifically, the RecBC pathway recombines two homologous chromosomes if at least one of them is linear (= has end(s)), the RecF pathway recombines two circular chromosomes, while the RecE pathway catalyzes phage recombination. Subsequent biochemical analysis (see 5.) revealed the two-fold meaning of the *recBC sbcA* and *recBC sbcBC* alternative pathways: 1) alternative ways of producing 3'-overhangs at double-strand ends; 2) alternative ways of loading RecA protein on these overhangs. In other words, in the wild type cells, only one way of double-strand end processing and RecA loading at this end is available (controlled by RecBC), while the RecF pathway loads RecA at single-strand gaps.

Recently, due to the central role of homologous recombination in repair of double-strand DNA breaks (also see below (4.3. and 5.2)), an entirely different approach — a color screen — was used to isolate recombination-affected mutants. Starting with *rdgB* or *dut* mutants, that have elevated levels of spontaneous chromosomal fragmentation due to, correspondingly, hypoxanthine-DNA or uracil-DNA incorporation and excision, secondary mutations were isolated that made the original single mutants inviable (synthetically lethal with the *rdgB* or *dut* defects) (204, 324). Among such RdgB- or Dut-dependent mutants, there were multiple hits inactivating all the known six genes of recombinational repair of double-strand breaks in *E. coli*: *recA*, *recB*, *recC*, *ruvA*, *ruvB* and *ruvC* (204, 324). The

prediction is that, if performed in the *recBC sbcA* or *recBC sbcBC* backgrounds, such a screen should yield all other known *rec* mutants, as well as (hopefully) some still unknown ones. As an approach, identification of synthetic lethal mutant combinations shows its power in unraveling complicated pathways of the DNA metabolism around recombinational repair (270, 357), although care should be exercised with interpretation of quadruple and quintuple mutants, as the comfort zone of typical human thinking is limited to three dimensions.

3.2. Substrate analysis

Another level of complexity of homologous recombination in *E. coli* was revealed when the defect (or its absence) of the isolated mutants in conjugational recombination was compared with their defect in plasmid recombination (both in the otherwise wild type background). The idea behind such a comparison is to change the substrate format from linear-by-circular for conjugational recombination to circular-by-circular for plasmid recombination. This substrate analysis revealed that some of the mutants, like *recA*, *recG* and *ruv*, were equally defective in both types of recombination, while some other mutants, like *recD*, *recN* and *recQ*, were wild type in both (reviewed in (39, 207, 303, 304)). The interesting mutants showed defect with one pair of substrates, while no defect with the other pair (Table 3). To illustrate the main results of this analysis, let us first make Table 3 clearer by 1) eliminating the mutants with no effect in the otherwise wild type strain in either format (*recD*, *recE*, *recN*, *recQ* and *recT*); 2) setting aside the *recA* mutants, which show the strongest effect in either format; 3) grouping together mutants with a strong effect in one format, but no effect in the other (*recB* and *recC* versus *recF*, *recJ*, *recO* and *recR*); 4) grouping together mutants with weak effects in either format (*recG* and *ruvABC*).

Clarified in this way, the results of substrate analysis suggest that: 1) RecA catalyzes both conjugational and plasmid recombination — likely by being responsible for the critical step in both processes (homology-guided strand exchange?); 2) RecB and RecC catalyze conjugational, but not plasmid recombination; 3) RecF, RecJ, RecO and RecR catalyze plasmid, but not conjugational recombination; 4) RecG and RuvABC are important, but not critical, for both conjugational and plasmid recombination. Since we have an idea about the nature of the substrates, we can conclude that the RecBC pathway catalyzes (RecA-mediated) exchanges between two DNAs if at least one of them has free ends (like the linear subchromosomal piece after conjugation), while the RecFJOR pathway catalyzes (RecA-mediated) exchanges between chromosomes without ends, for example, between two circular plasmids. We can also say that both RecG and Ruv functions help recombination, but the specificity of their action is unclear. However, since these mutants are insensitive to the substrate configuration, we can tentatively conclude that both RecG and Ruv functions do not recognize the initial recombination substrates (this recognition is, apparently, the function of RecBC and RecFJOR), but work at a later phase of the reaction.

The results of this idealized substrate analysis look convincing and satisfying, but the real life situations are usually more complicated and harder to interpret, stressing the importance of basing one's interpretations in this analysis on the precise information, instead of assumptions, about the format of the substrates. For example, in the Konrad system that detects events between two distant inverted repeats in the chromosome (147), recombination

depends on RecBC, rather than on RecF (360), suggesting that it is due to linear-by-circular type of events, rather than the assumed circular-by-circular ones. In a different chromosomal recombination system, again based on distant repeats, recombination depends on both RecBC and RecF, suggesting that the format of this reaction can be either linear-by-circular or circular-by-circular, depending on the situation (90). Finally, deletion formation at tandem repeats in the chromosome happens at unexpectedly high frequencies in the absence of RecA (285), forcing to classify this recombination as “homology-driven”, rather than “homologous”. The generic mechanism of such homology-driven recombination is single-strand annealing (reviewed in (160, 163)).

3.3. Epistatic analysis

Epistatic analysis involves combining in a single organism two mutations affecting the same process and monitoring the resulting phenotype; its application to sorting out DNA repair mutants in *Saccharomyces cerevisiae* is considered classic (28, 55, 91, 110). “Epistasis” means “covering over”, and originally epistatic analysis was designed to find genes acting together in the same pathway by looking for double mutants with a phenotype of one of the single mutants. Remarkably, epistatic analysis turned out to have more distinguishing power than at first thought, because it can also reveal distinct pathways within one broad mechanism. Suppose there are two mutants, each showing a 30% decrease of some measurable phenotype. The three kinds of epistatic interactions are: 1) the double mutant possesses the phenotype of the single mutants (still shows only 30% drop), — this classic epistasis suggests that the two genes work in the same pathway; 2) the double mutant shows an “additive” (actually, multiplicative) effect (50% decrease), — the two genes must be working in separate pathways, and there are more functional pathways left; 3) the double mutant shows a synergistic effect (99% down) — there are only two pathways, and the two mutations inactivate both (55, 110).

To see how epistatic analysis works for *E. coli* mutants in homologous recombination, let us first change the readout from recombination deficiency index in the two substrate configurations to something more general. As already mentioned, mutants in homologous recombination are sensitive to DNA damage, for example, to ultraviolet (UV) irradiation. If we take determinations of UV sensitivity of double mutants in comparison with UV sensitivity of the corresponding single mutants, for example, from papers of Lloyd and colleagues (99, 184, 187, 189, 192, 210), we can generate a matrix. To keep it simple, let us take a particular low dose of UV (say, 10 J/m², which will be sublethal for wild type cells) and to approximate sensitivity of any given mutant to this dose to the nearest order of magnitude (Table 4).

Such epistatic analysis shows that in *E. coli*:

1. by themselves, most *rec* mutations decrease the resistance ~10-fold (the first column). Only *recA* mutations make cells very sensitive to UV. This suggests several pathways of recombinational repair, with RecA protein playing the key role.

2. To confirm the key role of RecA, we combine *recA* mutation with other mutants pairwise (the second column): indeed, the *recA* mutant sensitivity is obtained for all double mutants => therefore, *recA* must control the limiting step common to all pathways of recombinational repair.

Combining all other mutations pairwise produces the following picture:

- 3 *recB* mutation does not change UV sensitivity when combined with *recC* or *ruv* mutations, => RecB protein works together with the corresponding proteins.
- 4 *recB* mutant becomes quite UV-sensitive if combined with *recG* and *recJ* mutations and extremely UV-sensitive if combined with *recF*, *recJ*, *recO* and *recR* mutations, => the RecFJOR group defines a pathway alternative to the RecBC-Ruv pathway, while RecGJ group may compensate for the *recB* defect in some way.
- 5 *recC* mutant combinations confirm the *recB* analysis.
- 6 *recF* mutation does not change UV sensitivity when combined with *recG*, *recJ*, *recO* and *recR* defects, but it makes cells more UV-sensitive if combined with the *ruv* defect and extremely UV-sensitive when combined with *recB* or *recC* defects, => RecFGJOR group works together and is separate from the RecBC group, while the Ruv function may play a compensatory role.

Now we again see the pattern of RecBC versus RecFJOR pathways, but the situation with RecG and Ruv is less clear, because although they both group with a particular pathway, their combinations with the other pathway have only moderate effects. However, when the *recG* and *ruv* mutants are combined together, an extremely sensitive double mutant is produced again! In summary, the results with *recG* and *ruv* mutants show that RecG and Ruv proteins 1) work at the stage of recombinational repair that is different from the stage at which RecBC and RecFOR work; 2) define the two alternative pathways at that other stage; 3) the early RecBC pathway preferentially feeds into the late Ruv pathway, while the early RecFOR pathway preferentially feeds into the late RecG pathway (although there is also a significant cross-feeding between the early and the late pathways).

If we combine the results of epistatic analysis with the results of the substrate analysis above, we arrive at the tentative formal scheme of homologous recombination in *E. coli* (Fig. 13):

1. *recA* is the control gene of HR, catalyzing the central reaction of the process, which is the formation of recombinational intermediate.
2. RecBC and RecFOR complexes define the two alternative early pathways, working before the RecA-promoted stage, most probably to assist RecA.
3. RuvABC and RecG enzymes represent the two alternative late pathways, working after the RecA-promoted stage, most probably by resolving recombination intermediates (for example, like in Fig. 3 and 4).

4. Evolution versus Survival

Homologous recombination may be important for evolution, but 1) it is stimulated by DNA damage; 2) although DNA damage does induce cellular (SOS) response, homologous recombination is stimulated directly by DNA lesions, rather than by overproduction of recombination functions as a part of the SOS response; 3) both homologous recombination and SOS response are induced by one-strand DNA damage after it has been aggravated by DNA replication, apparently spreading to the opposite DNA strand and becoming two-strand DNA damage; 4) recombination mutants are sensitive to DNA-damaging treatments and have reduced viability. Therefore, the physiological function of homologous recombination is to repair two-strand DNA lesions, which are, in effect, “chromosomal lesions”.

4.1. Stimulation of homologous recombination by DNA damage

The mechanism of homologous recombination proposed by Holliday (121, 260) (1.2.) provides a framework for thinking about the configuration of recombination intermediates and their resolution. However, the mechanism of Holliday does not provide the reason *why* two DNA duplexes should exchange strands in the first place and *how* the position for such an exchange is selected. In fact, with coincident nicks initiating the exchange, the original Holliday scheme is a perfect description of what we know about *site-specific* recombination (100), rather than homologous recombination. Then how and where is homologous recombination initiated?

To answer this question, one can examine what *stimulates* homologous recombination, assuming that recombination-stimulating conditions mostly increase the frequency of spontaneous recombination-inducing events, rather than generate novel classes of such events. Soon after homologous recombination was discovered in *E. coli*, it was found to be stimulated by DNA-damaging treatments (43, 132). Stimulation of homologous recombination in a variety of experimental systems by a variety of DNA damaging treatments is readily observed (65, 114, 203) and is documented beyond doubt (reviewed in (120)). The genetic requirements of the DNA damage-induced recombination are the same as those of spontaneous recombination ((111, 242), reviewed in (120)), implying that spontaneous recombination (i) has the same mechanisms as recombination induced by DNA damage; (ii) is itself induced by spontaneous DNA damage. Most DNA lesions affect only one DNA strand at any point; such “one-strand” DNA lesions are efficiently removed by one of the several excision-repair systems (reviewed in (89); also see Eco-Sal III Module 7.2.4. DNA Damage Reversal and Excision Repair). Since such repair would obviate any need for remedial interaction between homologs, the observed stimulation of homologous recombination by one-strand DNA damage might reasonably be attributed to induction of a cell-wide hyper-recombinogenic state. In other words, recombination may be an indirect consequence of cell's reaction to DNA damage, rather than direct consequence of DNA damage repair.

It is well known that treatment of cells with one DNA damaging agent, followed by incubation in growth conditions, makes them better prepared to repair DNA lesions from subsequent exposures to different DNA damaging treatments (216, 255). This inducible

response to DNA damage, called the SOS response by Miroslav Radman (262), is best demonstrated by the effect of split-dose DNA damaging treatments, during which repair-proficient cells show better survival after a particular dose of DNA damaging treatment if the treatment is delivered in several sub-doses separated by short time intervals (77, 277). The SOS response (182, 262) includes increased expression of about 30 genes in the *E. coli* chromosome and decreased expression of some 20 more genes and operons (54, 85). The products of induced genes participate in DNA damage repair (removal of DNA lesions) and tolerance (when cells live with DNA lesions without removing them); the products of suppressed genes participate in metabolism of various carbon sources, as well as in cell division. The current SOS reviews include EcoSal III Modules 5.4.3. “The SOS Regulatory Network” and 7.2.8. “The SOS Response”.

The idea that DNA damage induces homologous recombination mostly via induction of a cell-wide hyper-recombinogenic state predicts that hyper-recombination mutants (“hyper-recs”) in *E. coli*, if isolated, should be affected in the regulation of recombinational enzymes. For example, overproduction of the RecA recombinase may reasonably be expected to increase the frequency of spontaneous strand exchange between otherwise intact homologous DNA duplexes, increasing HR, but it does not (146). In fact, no *recA* gene upregulation mutants were ever isolated as hyper-recs in *E. coli*. Instead, all of the several hyper-recombination mutants found to date, with a single exception, increase the level of endogenous DNA damage (147, 361) or chromosomal damage (in the shape of disintegrated replication forks) (285, 286), suggesting that, independently of whether recombination enzymes are overproduced or not, DNA lesions are required *directly* to stimulate recombination. The single exception are *uvrD* mutants (reviewed in (163)), which may potentially accumulate extra endogenous DNA damage because of the UvrD helicase involvement in nucleotide excision repair and mismatch repair pathways (reviewed in (89)), but which are also demonstrably hyper-rec for a different reason. UvrD helicase disassembles RecA filaments (see 5.3.) both in vitro (238, 329) and in vivo (34), so RecA filaments are stabilized in *uvrD* mutants, increasing the overall recombination. However, this exception does not challenge the rule, because even in the absence of UvrD recombination still initiates at the sites of DNA damage. Thus, no hyper-recombinogenic state can be induced in wild type cells without DNA damage. Also, if damaged DNA is introduced into cells, the increased recombination is detected only between chromosomes that share homology with the damaged chromosome (96, 267), further arguing against the idea of indirect induction.

4.2. The idea of replication-dependent DNA lesions and of their subsequent recombinational repair

The exquisite sensitivity to DNA damage of the first isolated recombination-deficient mutants (40, 109) suggested that *E. coli* uses homologous recombination as the only way to repair certain DNA lesions. Still, it could be argued that homologous recombination happens independently of the DNA lesions, with the survivors being the lucky few that inherited, after recombination, only the damage-free segments of the genome, as was originally proposed to explain the multiplicity reactivation phenomenon in bacteriophage (205). This idea predicts that the induction of homologous recombination by DNA damage should always be accompanied by a decrease in viability, with recombinants being more frequent

only among the surviving chromosomes. However, moderate doses of DNA damaging agents that would be lethal for recombination-deficient mutants, yet sublethal for recombination-proficient cells, do induce recombination in the latter (318). Also, in experimental systems where survival of the damaged DNA does not depend on the *rec* status of the host strain (for example, transformation with a chemically-modified plasmid), the extent of DNA damage-induced recombination (transfer of the plasmid allele to the chromosome) does so greatly (22, 203). Thus, it seems certain that repair by homologous recombination results from an active mechanism rather than from a random redistribution of lesions.

Although the mechanisms of formation and the final structure of lesions requiring recombination for repair are still debated (58, 152, 163, 234), the specificity of the inducible response provides a clue. When the original DNA damage affects only one strand of the DNA duplex (like UV), the inducible response to this damage requires DNA replication (283, 354). Similarly, induction of homologous recombination by treatments that affect only one strand of the DNA duplex requires replication of the damaged DNA (51, 178), suggesting that one-strand lesions spread to the opposite strands by replication, and the resulting two-strand lesions induce both the SOS response and homologous recombination. Accordingly, “recombinational repair” is thought to be recruited to repair two-strand lesions, because the individual DNA strands cannot be mended by one-strand (excision) repair in this case (Fig. 14A) (124, 163). To make repair possible, the strands of an affected duplex are aligned by homology with the strands of an intact homologous duplex and then exchanged to form the classic recombination intermediate (compare Fig. 14B with Fig. 2, right, or Fig. 4A). As a result, both lesions become single-stranded (Fig. 14C) and can now be removed from the hybrid duplexes by one-strand (excision) repair (Fig. 14D).

4.3. Sensitivity to DNA damage and decreased viability of *rec* mutants

The various *rec* mutants show varying degree of sensitivity to DNA damage, their relative sensitivities being dependent on the DNA damaging agent used (Table 5). UVC-light (the short-wavelength UV) generates mostly pyrimidine dimers within the same DNA strand and is considered a typical one-strand DNA lesion-inducing agent (89). Nalidixic acid interferes with the function of DNA gyrase, which apparently leads to induction of double-strand DNA breaks (225, 230), so DNA gyrase can be viewed as an agent that induces predominantly two-strand DNA lesions. Ionizing radiation generates mostly one-strand DNA damage (oxidized bases, nicks), but also produces a measurable amount of direct double-strand breaks (89, 334), so ionizing radiation can be viewed as an agent inducing both one-strand and two-strand DNA lesions. Generally, the stronger the defect in homologous recombination, the higher the sensitivity to DNA damage.

The increased sensitivity of *Rec⁻* mutants to these highly artificial exogenous DNA damaging treatments does not by itself prove an important adaptive role for recombination in DNA repair, especially if the natural niche of an organism keeps it protected against exogenous DNA damage. However, the natural life cycle of *E. coli* alternates between periods of stable growth with little DNA damage (in the gut) and periods of little growth with severe DNA damage (in natural waters) (163, 284), making the repair role of

homologous recombination highly relevant. Furthermore, all mutants deficient in homologous recombination (*recA*, *recBC recF*, *ruv recG*) have decreased viability even when grown in benign laboratory conditions (Table 5), suggesting that normal cellular metabolism generates endogenous DNA damage that requires recombination for repair and making it likely that homologous recombination ability is continuously selected for its role in repairing this otherwise lethal DNA damage. What kind of DNA damage can be so lethal?

5. Chromosomal lesions and their repair via homologous strand exchange

Homologous recombination is the only known way to faithfully repair chromosomal lesions — the most severe, incapacitating DNA lesions possible. Most chromosomal lesions are formed when the cell replicates the damaged template DNA. Since an unrepaired chromosomal lesion is a “kiss-of-death” for the cell, homologous recombination represents a critical DNA repair mechanism the cell relies upon when replicating damaged DNA templates. Chromosomal lesions come in two basic flavors, explaining why *E. coli* has two distinct pathways of homologous recombination (163). By their biochemical properties, the recombinational repair proteins can be subdivided into three teams: 1) the strand-exchange (catalysis) team, responsible for the central step in recombinational repair; 2) the specificity teams, responsible for chromosomal lesion recognition and for the licensing of RecA polymerization at these lesions; 3) the cleaning-up teams, responsible for removal of spent RecA filaments and DNA junctions from recombination intermediates.

5.1. Physical detection and classification of chromosomal lesions

Although *one-strand* DNA lesions do compromise the accuracy of genetic information and can interfere with the progress of individual replisomes, they neither block the overall chromosomal replication or segregation, nor do they threaten chromosome integrity. Besides, all one-strand DNA lesions are repairable by either direct DNA damage reversal or by excision repair (see EcoSal III Module 7.2.4. DNA Damage Reversal and Excision Repair). In contrast, *two-strand* DNA lesions cannot be repaired by excision, because there is no intact template within the affected duplex segment. At the same time, two-strand DNA lesions, like double-strand DNA breaks (DSB), cannot be left unrepaired either, because of their lethal consequences for the cell (Fig. 15): 1) DSBs cause loss of genetic material due to DNA degradation from the exposed ends; 2) DSBs block replication of the chromosomal DNA, 3) DSBs interfere with segregation of the daughter chromosomes. Other types of two-strand DNA lesions share all or some of these lethal consequences with double-strand DNA breaks, — therefore, they all can be viewed as “chromosomal lesions”.

As is already clear from the example of the *two-ended* double-strand breaks (Fig. 15), not all two-strand lesions require DNA replication for their formation. Treatments with ionizing radiation produce double-strand breaks, which promptly reveal themselves by chromosome fragmentation (23, 98) and render chromosomes susceptible to degradation. Treatments with bifunctional alkylating agents result in DNA that can be rapidly renatured after experimental denaturation, suggesting *interstrand crosslinks* (264). Such crosslinks completely block DNA replication in their locality and prevent subsequent chromosomal segregation. Because double-strand breaks and interstrand crosslinks affect both DNA strands opposite each other,

but arise independently of DNA replication, they can be classified as direct chromosomal lesions.

Different kinds of chromosomal lesions form at replication forks that are trying to traverse single-strand DNA lesions (56, 233). In excision-repair-deficient mutants, single-strand gaps are found in DNA strands synthesized after UV-irradiation (272). The average size of the gaps is half the length of Okazaki fragments (131), while the number of gaps (at low UV doses) is similar to the number of original UV lesions (272). The gaps are thought to form when replication forks traverse pyrimidine dimers in template DNA (Fig. 4B) (124). Such gaps should form naturally in the lagging strand, due to the multiple priming events there; however, since all nascent DNA in vivo is first synthesized as Okazaki fragments (5, 249), the same gaps should also form in the leading strand. Interestingly, in vitro reinitiation downstream of the lesion in the leading strand is robust, even though in the absence of template strand lesions the leading strand is synthesized continuously in this system (113). Also, the demonstration that DNA polymerase III holoenzyme carries three catalytic assemblies makes it possible that replication forks shed individual replisomes (catalytic assemblies) that are stalled at lesions (229). In summary, the available evidence suggests that, when a replication forks encounters a non-coding lesion in one of the template strands, it reinitiates downstream, leaving behind single-strand gaps opposite the lesions (Fig. 16, left) (further reviewed in (163, 353)). These so-called *daughter-strand gaps*, if left unrepaired, preclude subsequent DNA replication, because both DNA strands now have lesions that would block the replisome progress. Daughter-strand gaps represent “blocked single-strand gap”, which is one general type of two-strand (chromosomal) lesions.

Events after UV-irradiation in *uvr*⁺ cells, which efficiently excise UV-induced pyrimidine dimers, reveal the other class of replication-dependent chromosomal lesions. In such cells, chromosomal DNA is fragmented; this fragmentation depends both on the ongoing excision repair and on DNA replication (reviewed in (163)). It is proposed that replication forks in these cells collapse when they run into transient excision gaps (Fig. 16, right) (107, 165). When both replication forks of a bubble collapse, this generates a linear chromosome with redundancy at the ends or a circular chromosome plus a subchromosomal fragment. The lesions in this case come in the format of a *one-ended* double-strand break.

Daughter-strand gaps and collapsed replication forks are replication-dependent chromosomal lesions and as such look quite different from direct chromosomal lesions — two-ended (direct) double-strand breaks and interstrand crosslinks. However, the four lesions can be grouped into two major classes. When a cell tries to repair a crosslink, it first excises one-strand portion of it, but the repair is then stalled, because the second strand cannot be used as a template to resynthesize the excised DNA strand (48). Therefore, excision repair converts interstrand crosslink into a persistent, *blocked* single-strand gap and, in this quality, the first intermediate in interstrand crosslink repair resembles daughter-strand gaps (Fig. 17).

In contrast to a direct, two-ended double-strand break, replication fork collapse generates one-ended double-strand end. However, a double-strand break can be viewed as a special case of a more general replication fork collapse involving two replication forks converging

on a nick, an event that generates two double-strand ends. Thus, we can group the four chromosomal lesions as follows: interstrand crosslinks and DSGs into the group of blocked single-strand gaps (one strand interrupted), while DSBs and RFCs into the group of double-strand ends (two strands interrupted) (Fig. 17). Finally, to complete the classification, there are also chromosomal lesions that do not involve any DNA damage and yet they still interfere with chromosomal replication and/or segregation. The direct lesions of this kind are chromosomal dimers (296), while the replication-dependent lesions without DNA damage are (hypothetical) locked replication forks (102, 168) (Fig. 17).

5.2. The inferred pathways of recombinational repair

In *E. coli*, chromosomal lesions (two-strand DNA lesions) that involve DNA damage are repaired by homology-guided strand exchange between sister chromatids. The evidence in support of this notion comes in three flavors (reviewed in (163)). First, physical connections between parental and daughter strands, associated with lesion repair, can be detected. Formation of such hybrid strands is the most direct physical evidence of strand exchange; such evidence is available for daughter-strand gap repair (273) and for the repair of crosslinks (47). Second, repair of chromosomal lesions is not observed in *recA* mutants. Such evidence is available for double-strand breaks (150), daughter-strand gaps (305) and crosslinks (46), as well as for the yet-to-be-physically-confirmed lesions such as collapsed replication forks (reviewed in (159, 161)). Third, DNA damage stimulates homologous recombination (4.1.), although the structure of chromosomal lesions in this case is not specified. Also, resolution of recombinational repair intermediates between sister duplexes in the *E. coli* chromosome is expected to generate sister-chromatid exchanges, detected as chromosome dimerization; formation of dimeric chromosomes is stimulated in *E. coli* mutants with increased DNA damage and is eliminated in single *recA* or in double *recB recF* mutants (310, 311).

As argued above (5.1.) the final configuration of chromosomal lesions with associated DNA damage belongs to one of the two basic types (Fig. 17): either a blocked single-strand gap (a daughter-strand gap or the product of an attempted excision repair of a crosslink) or a double-strand end (a pair of ends in the case of a direct double-strand break, a solitary end in the case of replication fork collapse). Double-strand end repair is not observed in *recA* or in *recBC* mutants (150, 281), suggesting that it is catalyzed by the RecBC pathway of homologous recombination (3.). Similarly, repair of blocked (persistent) single-strand gaps is not observed in *recA* or *recFOR* mutants (269, 305, 326), suggesting that it is catalyzed by the RecFOR pathway (3.). The central role of RecA in this repair suggests a common critical step. Both types of lesions disappear with normal kinetics in *recG* or *ruv* mutants, indicating that the products of these genes are not involved in repair of broken strands *per se*. However, both *recG* and *ruv* mutants have a specific problem after DNA damage: their nucleoids cannot untangle, as if kept together by unresolved recombinational repair intermediates (129, 130), consistent with the postulated late role of the RecG and Ruv enzymes in recombinational repair reactions, specifically in resolution of DNA junctions.

A word of caution here: although the proposed individual pathways are supposed to cover all possible chromosomal lesions, they most likely do not. Some complex DNA lesions may

require combination of several pathways to complete repair. For example, repair of interstand crosslinks requires cooperation of at least nucleotide-excision repair (see Eco-Sal III Module 7.2.4. DNA Damage Reversal and Excision Repair) and both RecBC and RecFOR pathways of recombinational repair (41, 47, 48, 300). In general, repair of complex DNA lesions or clustered DNA lesions (tight groups of ones-strand lesions) is a barely explored area.

5.3. The mechanisms of recombinational repair

Plausible schemes for the two recombinational repair reactions are shown in Fig. 18. In both cases, the affected DNA region, with the help of RecA protein, invades the homologous region on the intact (sister) chromosome. After the resolution of DNA junctions and repair of one-strand lesions, the replication is restarted, either to fill the gaps or to resume a replication fork. It should be stressed, though, that, while genetic analysis is powerful enough to figure out the entire pathway of a particular reaction in terms of genes and hypothetical reaction stages (Fig. 13), it must rely on biochemical characterization of reactions, catalyzed by the corresponding proteins *in vitro*, to translate a formal pathway scheme into a detailed mechanism. The biochemical characterization of the Rec and Ruv proteins showed the following (see (163, 168) for more in-depth discussion):

RecA protein polymerizes into spiral filaments on ssDNA in the 5'→3' direction, making RecA filaments more stable on the 3'-ends of linear ssDNA than on the 5'-ends (180, 263, 292). Such a ssDNA-RecA filament is the homologous pairing and strand exchange entity (reviewed in (148, 265)) that catalyzes single-strand invasion into a circular duplex (63, 226, 297). Two-strand DNA lesions (in which DNA strands are interrupted) are always associated with ssDNA or are processed to become associated with ssDNA (168), so this ssDNA invasion catalyzed by RecA is precisely what is needed to form a recombinational repair intermediate. Thus, RecA should be the central activity of recombinational repair, catalyzing the all-important strand exchange step, which is fully consistent with the conclusion of genetic analysis. RecA filaments also serve as a scaffold for binding and activation of a peculiar cryptic autoprotease called LexA (122, 181), which is a repressor for the SOS regulon, critical for the overall resistance of *E. coli* to DNA damage (see Eco-Sal III Module 5.4.3. The SOS Regulatory Network, and Module 7.2.8. The SOS Response). For in-depth discussion of the *in vitro* properties and structure of RecA, please consult section 2 of the Eco-Sal III Module 7.2.7. "Homologous Recombination — Enzymes and Pathways".

Since *in vitro* RecA polymerizes on ssDNA even in the presence of SSB protein (under certain conditions) (149, 172, 323), it may be unclear what keeps RecA off ssDNA regions, which are always present at replication forks, in the absence of DNA damage *in vivo*? There is an argument, based on the intracellular nucleotide and Mg^{2+} concentrations, that normal physiological conditions are suboptimal for RecA polymerization in the presence of SSB (158). There is also a kinetic argument that *in vivo* RecA cannot polymerize on ssDNA that does not need recombinational repair (283) because the kinetics of RecA polymerization on SSB-complexed ssDNA *in vitro* has a lag, while the polymerization itself, once started, is fast (61, 323). This bi-phasic property of RecA polymerization, or its stimulation by increasing Mg^{2+} concentration, suggest the existence of a limiting step, — the nucleation

event. In other words, in the cell, RecA apparently needs to be *licensed* to polymerize on ssDNA. What is the nature of this license and does it also provide any specificity for RecA polymerization? To answer these questions, we should keep in mind that the enzymes catalyzing the early phase of recombinational repair should not only promote RecA polymerization, but should also be able to recognize specific chromosomal lesions (that is, possess substrate-specificity).

Of all the proteins in *E. coli* cells, RecBCD complex has the highest affinity toward double-strand DNA ends (on the order of 1 nM, or 0.1 ends per cell) (266, 316), making it the first enzyme to bind to the ends of a double-strand break. Besides securing the ends, the RecBCD enzyme has several other relevant activities in vitro: 1) DNA end-specific helicase; 2) ATP-dependent dsDNA and ssDNA exonuclease; 3) stimulator of RecA polymerization on a particular DNA strand (reviewed in (73)). Circular duplex DNAs or SSB-complexed circular single-stranded DNAs are completely resistant to the action of RecBCD. After binding to a double-strand DNA end, RecBCD uses its helicase/exonuclease activities to generate a 3'-overhang of ssDNA, which is instantly complexed by SSB. RecBCD eventually stops and promotes RecA polymerization on the generated ss-overhang by providing a nucleation push for RecA and thus aiding it in the displacement of SSB (reviewed in (73)). For in-depth discussion of the in vitro properties and structure of the RecBCD enzyme, please consult section 3 of the Eco-Sal III Module 7.2.7. "Homologous Recombination — Enzymes and Pathways".

RecF, RecO and RecR proteins in vitro can bind ssDNA, as well as duplex DNA (reviewed in (59)). In addition, RecO and RecR proteins stimulate RecA polymerization on SSB-complexed ssDNA. Lastly, both RecF and RecO bind RecR protein, but not each other (reviewed in (59)). As a group, if not a single complex, RecF, RecO and RecR proteins help RecA to polymerize on DNA with ss-gaps (239). It has been speculated that RecF has affinity to DNA with ss-gaps in vivo, and, using its affinity to RecR, it targets RecOR proteins to these gaps (163). The ss-gaps in vivo are, of course, complexed by SSB, but RecOR proteins provide a nucleation signal for RecA (reviewed in (59)), so RecA displaces SSB on ssDNA gaps, if licensed by RecFOR. For in-depth discussion of the in vitro properties and structures of RecF, RecO and RecR proteins, please consult section 4 of the Eco-Sal III Module 7.2.7. "Homologous Recombination — Enzymes and Pathways".

The late recombinational repair functions, encoded by *recG* and *ruv* genes, can be better understood as the opposites of the early functions, RecBCD and RecFOR. Whereas the two main roles of the early functions are (i) to recognize specific chromosomal lesions; (ii) to license RecA polymerization on these lesions, the two main roles of the late functions are (i) to recognize and remove generic recombination intermediates; (ii) to disassemble RecA filaments associated with these recombination intermediates (discussed in (163, 168)).

Since the most basic recombinational repair intermediate should contain Holliday junctions, the RecG and Ruv proteins were expected to have affinity to Holliday junctions and to catalyze reactions with them. The *ruv* locus turned out to be a complex one, comprising three genes: *ruvA*, *ruvB* and *ruvC* (293, 298). RuvA protein in vitro indeed binds completely double-stranded four-way DNA junctions (Holliday junctions) and changes their

conformation from the folded one (difficult to branch-migrate) to the square planar one (easy to branch-migrate) (reviewed in (339)). RuvB in vitro forms hexameric doughnuts around duplex DNA, interacts with RuvA and, while holding on to RuvA, can “pump” duplex DNA through the central cavity of the hexameric ring (reviewed in (339)). RuvB is a distant relative of hexameric helicases, but its “pumping” action actually anneals DNA strands, separated at the Holliday junction, rather than unwinds DNA duplexes. RuvAB complex also depolymerizes the RecA filaments, associated with Holliday junctions (2). RuvC is another protein that binds Holliday junctions, again changing their conformation to the planar one, and then symmetrically cleaves a pair of opposite strands at special sites to “resolve” the junctions (reviewed in (339)). RuvABC, working as a single complex, “resolvasome”, binds, isomerizes, protects, branch-migrates and resolves Holliday junctions (reviewed in (164, 166, 339)). For in-depth discussion of the in vitro properties and structures of RuvA, RuvB and RuvC proteins, please consult section 5 of the Eco-Sal III Module 7.2.7. “Homologous Recombination — Enzymes and Pathways”.

RecG helicase binds on one side of three-way or four-way DNA junctions and branch-migrates them by effectively “squeezing out” the DNA on the opposite side of the junction (reviewed in (163)). RecG can also depolymerize RecA filaments associated with DNA junctions (340). Genetic data suggest that RecG, like RuvABC, participates in the DNA junction removal (184), but the mechanisms of its action are poorly understood (228, 271). One idea is that RecG works on the double Holliday junctions to migrate them toward each other until they come together and are, thus, eliminated (“dissolved” into each other) (163). Such “dissolution” will also require the action of DNA gyrase between the two junctions (or Topo IV outside), to remove the DNA coils. To learn more about the in vitro properties and structure of RecG helicase, please consult section 6 of the Eco-Sal III Module 7.2.7. “Homologous Recombination — Enzymes and Pathways”.

To summarize, the recombinational repair proteins can be subdivided into three groups: 1) The strand-exchange proteins — the catalysis team, responsible for the central step in recombinational repair. RecA in bacteria, assisted by SSB. 2) The enzymes recognizing chromosomal lesions, targeting them for RecA polymerization and licensing RecA polymerization on SSB-complexed ssDNA — the specificity teams. These include RecBC and RecFOR activities. 3) The enzymes removing spent RecA filaments and DNA junctions from hybrid duplexes — the cleaning-up teams. These include the RuvABC resolvasome and the RecG helicase.

Conclusion

Homologous recombination in *E. coli* is a complex phenomenon with clear evolutionary implications and an important everyday metabolic purpose. For the students of homologous recombination, both *E. coli* and *Salmonella* offer solid understanding of the core events, several formats of elaborate experimental systems to address its various aspects, well-developed genetics with several pathways, and unparalleled biochemistry. This chapter strived to cover all important aspects of homologous recombination in *E. coli* outside the biochemical and structural aspects of it, which receive separate in-depth coverage in the

recombinational repair chapter by Michel and Leach (Eco-Sal III Module 7.2.7. Recombinational Repair — Enzymes and Pathways).

Since the study of homologous recombination laid the groundwork for the subsequent successful study of recombinational repair, it may be unclear why biochemistry of homologous recombination in *E. coli* should be discussed in a separate chapter and under a different title, apart from its phenomenology and genetics. The answer comes naturally from considering the phenomena of “homologous recombination” *vis-à-vis* “recombinational repair”. “Recombinational repair” in bacteria is a particular metabolic transaction, with specific substrates (non-functional chromosomes) and products (functional chromosomes), defined enzymatic steps and important outcome for the cell. An ideal recombinational repair transaction is genetically silent, just like an ideal DNA replication round, a part of which the former actually is. In contrast, “homologous recombination” is always a result, rather than a specific process, a peculiar and unintended consequence of sojourning of two homologous DNA sequences in the same cell, a rearrangement of the genetic material that might advance the lineage in the evolutionary race, but more likely will be without effect or will have deleterious consequences due to changes in the chromosomal structure. Studies of homologous recombination illuminate the future of recombinational repair, but do not yield the physiologically-relevant mechanisms of DNA metabolism the latter is famous for. The two indeed have different purposes, practice different philosophies and provide different insights, — therefore they deserve separate dedicated chapters.

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Preamble

The introduction (part 1) to this module is written with a beginner graduate student in mind: it starts with a thorough definition of homologous recombination in its relation to other types of recombination, spends quality time on recombination intermediates, goes through the basic types of rearrangements due to homologous recombination, explains the peculiarity of mapping by recombination in prokaryotes and finally briefly goes over recombination detection. Homologous recombination aficionados, who will likely find such an introduction unnecessarily trivial, should start with part 2.

Fig. 1.

Illustrations to the search for definition of homologous recombination. DNA duplex is shown as a single line. The straight lines (whether open or filled) represent homologous DNA, the wavy and straight lines represent heterologous DNAs. A, B, a, b, Y and Z represent “markers” — DNA sequences that confer distinct phenotypes. “A” and “a” represent alleles of the same gene. Explanations are in the text.

Fig. 2.
Theoretically-conceivable pathways of formation of homologous recombination intermediate with two Holliday junctions.

Fig. 3.
Resolution of a single Holliday junction.

Fig. 4.

Resolution of the double Holliday junction recombination intermediate. Each diagonal pair of resolution cuts is numbered either “1” or “2” for each junction. If the two junctions freely isomerize and are resolved independently of each other, four outcomes of the resolution are expected. In two of the outcomes, the chromosome arms will be exchanged, resulting in recombinant chromosomes. **A.** A joint molecule with two junctions as shown in Fig. 2, right. **B.** The same joint molecule isomerized so as to show both junctions in the open planar configuration (258). **C.** The four resolution outcomes, numbered according to the resolution options realized at the left and the right junctions. Note that two outcomes (1-1 and 2-2) produce chromosomes with recombinant shoulders, while the other two outcomes (1-2 and 2-1) produce chromosomes with parental shoulders.

Fig. 5.

Homologous recombination can generate insertions or deletions. **A.** Recombination between a circular and a linear chromosomes by the region of shared homology inserts the circular chromosome into the linear one. **B.** Recombination between two directly-oriented regions of homology on the same chromosome leads to the loss of genetic information (deletion) in the form of a circle.

Fig. 6.

Homologous recombination can generate inversions, unequal sister chromatid exchange, as well as dysfunctional chromosomes. **A.** Recombination between inverted repeats within the same chromosome generates an inversion. **B.** Recombination between sister chromatids carrying direct repeats can lead to unequal sister chromatid exchange (deletion in one chromatid, duplication in the other). **C.** Recombination between sister chromatids carrying inverted repeats generates dysfunctional chromosomes and most of the times will result in cell death.

Fig. 7.

Crossing-over versus gene conversion: classification of detectable events. The two parental chromosomes are shown at the top. Alternatively, these could be two repeated sequences within the same chromosome. Although the two chromosomes (or repeats) are essentially homologous over their entire length, they are assumed to have enough markers (easily scorable disagreements in their primary DNA sequences) to facilitate fine mapping of the recombination events and conversion tracts. The four major types of recombination events, all results of alternative processing of a single recombination intermediate as in Fig. 2 (right) and Fig. 4A, are presented.

Fig. 8. Chromosomal mapping in eukaryotes and in bacteria: peculiarities of the life cycles. Explanations in the text. **A.** Mapping based on segregation frequencies in eukaryotes. **B.** Growth of a unicellular eukaryote with periodic sexual events: either syngamy or meiosis. Circles denote gametes ($1n$ cells), ovals denote zygotes ($2n$ cells). **C.** Strictly clonal growth in the majority of bacteria. **D.** Mapping based on co-inheritance frequencies in bacteria.

Fig. 9.

Detriment of single exchanges in bacterial chromosomes and permissibility of double exchanges. **A.** A single exchange with a linear subchromosomal fragment introduces unprotected ends into the chromosome leading to chromosome degradation. The chromosome is shown linear, with telomere-protected ends, which does not change the argument. **B.** The same situation as in “A”, but with two exchanges, which leave the chromosome physically intact, even though genetically different. **C.** A single exchange between two circular chromosomes creates a chromosomal dimer, which will kill the cell if left unresolved. **D.** The same situation as in “C”, but again with two exchanges, that keep the two recombining chromosomes monomeric and, therefore, functional. **E.** The double exchange from “B” is shown with markers to illustrate a better applicability of coinheritance, rather than segregation, to quantify crosses in systems in which only double exchanges survive.

Fig. 10.

The difference between a full single crossover event and a half-crossover event. In this case, both strands of participating DNA duplexes are shown. One homologous duplex has open strands, the other homolog has filled strands.

Fig. 11.

The three basic topological formats of recombination reaction. In spite of the argument of Fig. 9, for the sake of clarity a single crossover is shown in all cases.

Fig. 12.

Formation of the head-to-head plasmid dimer, mediated by inverted repeats. This is, basically, a full-chromosome version of Fig. 6C.

Fig. 13.

The formal genetic scheme of homologous recombination in *E. coli*. The scheme is based on the result of the substrate analysis and of the epistatic analysis. The chromosomal duplex DNA is shown as a single line, with the exception of the double Holliday junction, which is shown double-stranded. Explanations in the text.

Fig. 14.

The idea of two-strand DNA repair. **A.** A DNA molecule with a two-strand lesion (small open rectangles in the filled duplex) is shown homologously aligned with an intact homolog (open duplex). **B.** The two sequences have exchanged strands, converting the two-strand lesion into a pair of one-strand lesions. **C.** Holliday junction resolution (the nicking of strands is shown in "B") separates the chromosomes from each other. **D.** Excision repair removes the one-strand lesions, completing the overall repair reaction. Note that if the black and white "parental" DNAs are homologs (carry genetically-scorable differences), rather than identical sisters, the resulting chromosomes may be detectably recombinant.

Fig. 15.

The lethal consequences of chromosomal lesions, exemplified by chromosomal fragmentation due to double-strand DNA breaks. The chromosome is shown by a double-line square. Yellow highlights in red frames: either double-strand breaks or their consequences. Brown circles with black arrows nearby, centromeres and the direction they are going. Blue arrows, normal cellular processes (chromosomal replication and segregation). Magenta arrows, chromosomal fragmentation. Red arrows, consequences of chromosomal fragmentation. **A.** Chromosome. **B.** Initiation of chromosomal replication. **C.** Initiation of sister chromosome segregation. **D.** Fragmentation of non-replicating chromosome. **E.** Fragmentation of replicating chromosome. **F.** Fragmentation of segregating chromosome. **G.** Gene loss due to DNA degradation from unprotected ends. **H.** Inability to replicate the chromosomal segment, separated from *oirC* by double-strand breaks. **I.** Inability to segregate chromosomal segment, separated from centromere by double-strand breaks.

Fig. 16.

The two basic types of replication-dependent two-strand DNA lesions. A replication fork is moving from left to right along the template DNA with unrepaired one-strand lesions. The template on the left contains a non-coding lesion (T=T, thymine dimer), while the template on the right has a single-strand interruption. Replication fork encounter with a non-coding lesion generates a daughter-strand gap (left), while replication fork encounter with a nick generates a one-ended double-strand break (= double-strand end, right)

# of strands interrupted	Direct	Replication-dependent
2	 two-ended double-strand break	 replication fork collapse at nick one-ended double-strand break
1	 inter-strand crosslink persistent ssDNA gap	 non-coding lesion in template strand daughter-strand gap
0	 single crossover chromosomal dimer	 template switching locked replication fork

Fig. 17. One possible classification of chromosomal lesions. This classification is based, on the one hand, on whether formation of these lesions depends or not on DNA replication (“direct” versus “replication-dependent”) and, on the other hand, on the number of DNA strands interrupted in the final format of the lesion. In *E. coli*, the repair of chromosomal lesions with either one or two strands interrupted completely depends on recombinational repair. Chromosomal dimers are resolved by FtsK-XerCD/*dif* resolution system (site-specific recombination). Locked replication fork is still a hypothetical lesion, consistent with certain gross-chromosomal rearrangements, and whose (hypothetical) repair is likely to be dependent on homologous recombination.

Fig. 18.

The two pathways of recombinational repair in *E. coli*. On the left, the daughter-strand gap repair pathway is shown, while the double-strand end repair pathway is on the right. Stages are marked with enzymes that catalyze them.

Table 1

Comparison of various types of genetic recombination in respect of frequency, DNA requirements and the enzymes that catalyze these events.

Recombination type	Frequency	Requirements	Catalyzed by
1. Illegitimate	extremely low	Micro- or no homology	various DNA-processing enzymes
2. Transposition	very low (regulated)	Ends of the jumping element	Transposase
3. Homologous	low (when DNA damage is low)	Extensive homology	RecA, RecBC, RecFOR,...
4. Site-specific	high	A pair of short sites	Site-specific recombinase (integrase, invertase)

Table 2

Conjugation recombination deficiency indices for various *E. coli* mutants in homologous recombination in wild type, as well as in *recBC sbcA* and *recBC sbcBC* backgrounds. Instead of showing precise numbers, that can be found in the papers cited in section 3.1., the table shows magnitude of effects to expect from specific mutations in three recombinational repair proficient backgrounds (WT, *recBC sbcA* and *recBC sbcBC*).

Mutant	Background		
	WT	<i>recBC sbcA</i>	<i>recBC sbcBC</i>
Rec+	—	—	—@
<i>recA</i>	000	000	000
<i>recB</i> #	00	na	na
<i>recC</i> #	00	na	na
<i>recD</i> #	—	na	na
<i>recE</i>	—	00	na
<i>recF</i>	—	00	00
<i>recG</i>	0	0	0
<i>recJ</i>	—	0	0
<i>recN</i>	—	0	00
<i>recO</i>	—	00	00
<i>recQ</i>	—	0	0
<i>recR</i>	—	00	00
<i>recT</i>	—	00	*
<i>priA</i> *	0	(0)	(0)
<i>ruv</i>	0	0	0

— - no effect

@ - although the final recombination frequencies are 50% of the wild type in the *recBC sbcBC* mutants, the kinetics of recombinant formation is extremely slow compared with wild type (194).

na - not applicable (mutant)

0 - approximately one order of magnitude down

00 - approximately two orders of magnitude down

000 - approximately three orders of magnitude down

- the RecBCD proteins work as a single complex

* - PriA is a replication restart function, connecting recombinational repair with DNA replication.

Table 3

Quantitative illustration of substrate analysis: conjugational (linear $\times$ circular) versus plasmid (circular $\times$ circular) recombination deficiency indices for various *E. coli* single mutants in homologous recombination. Again, instead of showing precise numbers, that can be found in the papers cited in section 3.2., the table shows magnitude of effects to expect from specific mutations in the two substrate pair setups.

	linear $\times$ circular	circular $\times$ circular
Rec+	—	—
<i>recA</i>	000	00
<i>recB</i>	00	—
<i>recC</i>	00	—
<i>recF</i>	—	00
<i>recJ</i>	—	00
<i>recO</i>	—	00
<i>recR</i>	—	00
<i>recG</i>	0	0
<i>ruv</i>	0	0

Table 4

Quantitative illustration of epistatic analysis using resistance to UV-irradiation as a read-out. In the following matrix, the values show how many orders of magnitude mutants are more UV-sensitive than WT cells at a particular dose of UV (10 J/m²), sublethal for the wild type cells. Again, instead of showing precise numbers, that can be found in these papers (99, 184, 187, 189, 192, 210), the table shows magnitude of effects to expect from specific mutations or their pairwise combinations.

	WT	recA	recB	recC	recD	recF	recG	recJ	recO	recR	ruv
WT	1										
recA	1000	1000									
recB	10	1000	10								
recC	10	1000	10	10							
recD	1	1000	10	10	1						
recF	10	1000	1000	1000	10	10					
recG	10	1000	100	100	10	10	10				
recJ	10	1000	100	100	100	10	10	10			
recO	10	1000	1000	1000	10	10	10	10	10		
recR	10	1000	1000	1000	10	10	10	10	10	10	
ruv	10	1000	10	10	10	100	1000	10	100	100	10

Table 5

Viability of homologous recombination mutants and their sensitivity to different kinds of DNA damaging treatments. The doses of the DNA damaging treatments are in all cases sublethal for the WT cells (survival 80% or higher). (Disclaimer: The references were selected for mutant-to-mutant consistency and, in some cases, are neither original demonstrations, nor the wild type background. Moreover, since this is a compilation from different studies done in different laboratories at different times, the conditions and backgrounds are variable, limiting usefulness of this comparison to providing only the most general guidance.)

Mutation	DNA damaging treatment				Ref.
	UV ^a	NA ^b	IR ^c	Via ^d	
None (wild type)	0.8	0.8	0.8	1.0	N/A
<i>recA</i>	<0.0001	0.005	0.002	0.5	(98, 253, 344)
<i>recBC</i>	0.003	0.005	0.01	0.3	(98, 209)
<i>recD</i>	1.0			1.0	(189)
<i>recFOR</i>	0.05	1.0		0.8-1.0	(190, 192, 209)
<i>recG</i>	0.2		0.1	0.8	(187)
<i>recJ</i>	0.3			0.9	(209)
<i>recL (uvrD)</i>	0.001	0.005		0.6	(8, 185)
<i>recN</i>	0.5		0.5	1.0	(253)
<i>recQ</i>	1.0			1.0	(189)
<i>ruv</i>	0.002		0.1	0.6	(184, 250)
<i>priA</i>	0.02		0.02	0.1 ^e	(144)
<i>xerCD</i> or <i>dif</i>	1.0			0.6-0.9	(235)
<i>recBC recF</i>	0.00001	0.003		0.25	(191, 192, 209)
<i>ruv recG</i>	0.00001			0.25	(184)

^a Ultraviolet light sensitivity is shown as the ratio of survival of the mutant to the survival of the WT cells at the dose of 20 J/m², cells seeded on LB plates.

^b Nalidixic acid sensitivity (225) is shown as the ratio of survival of the mutant to the survival of the WT cells after incubation in LB with 100 µg/ml of the drug for one hour at 37°C.

^c Ionizing radiation sensitivity is shown as the ratio of survival of the mutant to the survival of the WT cells at the dose of 8 Krads.

^d Viability is measured as colony-forming units per volume of a rapidly-growing culture at a particular OD₆₀₀, divided by the same value for the wild type cells.

^e minimal media only.