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Interaction of the *Escherichia coli* host and phage λ: Role of the RecBCD-Gam complex

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Biochemical experiments have suggested that the Gam protein from phage λ inhibits all the catalytic activities of the RecBCD enzyme of *Escherichia coli*. The genetic data are at variance with these experiments. The RecBCD-binding Gam protein has profound effects on the physiology of phage λ and its host. These effects cannot be explained by the inhibitory action of Gam: the RecBCD-Gam complex apparently retains certain enzymatic activities. The mechanism by which its activities aid a substantial fraction of the phage to escape the *EcoK* restriction is proposed.

The RecBCD enzyme of *Escherichia coli* is a large, complex protein composed of three polypeptides (subunits) – RecB (≈135 kDa), RecC (≈125 kDa) and RecD (≈60 kDa). These subunits are coded for by *recB, recC and recD* genes (for recent reviews, see 1–5).

Bacteria with mutations in the *recB* or *recC* gene, or both, are deficient in genetic recombination and in DNA repair.[6–8] They display a "cautious degradation" phenotype, *i.e.* they show a less than normal breakdown of the damaged chromosomal DNA.[6,9] Likewise, they are unable to degrade the incoming foreign DNA, *e.g.* the DNA of phage T4 *2*.[10] Furthermore, the cultures of these mutants accumulate a large number of nonviable (residually dividing or nondividing) cells.[11]

In contrast to the *recB* and *recC* strains, bacteria mutated in *recD*, the gene for the small subunit of RecBCD enzyme, are fully viable.[12] *recD* cells are also recombination-proficient and resistant to ultraviolet light.[12] In these respects, they resemble the wild-type strain. Like *recB* and *recC* bac-

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teria, however, they are incapable of degrading foreign or damaged DNA[13,14] and of repairing certain types of the DNA lesions.[15,16]

The phenotype associated with mutations in the *recB* or *recC* gene is a consequence of elimination of all functions of the RecBCD enzyme. Biochemical experiments showed that this enzyme possesses a variety of activities. It acts as a DNA-dependent ATPase, DNA helicase and DNase.[17] RecBCD DNase cleaves single-stranded DNA endonucleolytically and degrades both single- and double-stranded DNA exonucleolytically. The RecBC enzyme, missing the RecD subunit, retains reduced but significant levels of helicase, endonuclease and ATPase activities.[15] This may explain why mutations in the *recD* gene have only moderate effects on the physiology of *E. coli*. RecBC enzyme, however, lacks both single-stranded and double-stranded DNA exonucleolytic activities.[15]

Absence of RecBCD exonuclease and the reduced helicase activity alter the response of *recD* cells to certain phages; for example, T4 *2* and λ *red gam* plate at high efficiency on the *recD* mutant host.[18] Because of the presence of the RecBCD enzyme, the growth of these phages is inhibited in wild-type bacteria.

The action of the RecBCD enzyme on phage growth may be considered as a host defense mechanism directed against foreign genetic material. This action complements the function performed by restriction endonucleases, such as *Eco*K (*e.g.*, Ref. 19). A number of phages can, at least to some extent, counteract the effects of both defense mechanisms. In this respect, the *gam* gene product of phage λ (Gam protein[20]) is of particular interest.

Gam protein binds to the RecBCD enzyme, thus forming the RecBCD-Gam complex.[21] In the absence of this binding, the RecBCD enzyme blocks the switch from early to late λ DNA replication.[22,23]

The early stage of replication is characterized by circular DNA intermediates. These intermediates are insensitive to the action of the RecBCD enzyme. During late replication, DNA concatemers (polymers of several DNA units) predominate. The concatemers, whose formation is inhibited by the RecBCD enzyme, are immediate precursors to the mature phage progeny. Hence, by interacting with the RecBCD enzyme and by enabling λ to undergo late replication, Gam protein plays an essential role in phage development.

The late stage of λ DNA replication can also occur in *recB* and *recC* cells.[22] This might be taken as an indication that Gam protein inhibits RecBCD enzyme, thus converting *recBCD*$^+$ cells to RecBCD$^-$ phenocopies. Indeed, all known enzymatic activities of the RecBCD enzyme are inhibited by Gam protein.[21,24,25,26] However, the results of *in vivo* experiments are at variance with these biochemical data.

The *recBCD*$^+$ bacteria expressing Gam protein (due to the presence of the *gam*$^+$ plasmid or due to the transient induction of λ) are only partially

similar to *recB* and *recC* mutants. With respect to certain phenotypes, they bear much resemblance to wild-type cells. Also, there are some properties that distinguish them from wild-type bacteria as well as from the *recB* and *recC* mutants.

Examples of the effects of the RecBCD-Gam complex on the physiology of *E. coli* are given below.

Phage growth. Most phages (*e.g.*, T4, T7, λ) must protect themselves from the action of the RecBCD enzyme (see above). If unprotected, they can grow normally within the Gam-expressing *recBCD*$^+$ cells.[22,27] On the other hand, there are some phages (e.g., P1) whose development depends on the functional RecBCD enzyme. Within *recBCD*$^+$ cells, the growth of these phages is inhibited by the presence of Gam protein.[22,27] Thus, if phage growth is used as the criterion, Gam protein converts *recBCD*$^+$ bacteria to RecBCD$^-$ phenocopies.

DNA degradation. The presence of Gam protein within *recBCD*$^+$ cells strongly inhibits degradation of foreign or damaged chromosomal DNA.[27,28,29,30] Again, it seems that these cells are converted to RecBCD$^-$ phenocopies. (Mutations in the *recD* gene produce, however, the same effect.)

Bacterial growth. As already mentioned, the large populations of nonviable cells accumulate in the cultures of *recB* and *recC* strains. The growth rate of these cultures is, therefore, greatly decreased. Wild-type bacteria expressing Gam protein do not behave like *recB* and *recC* strains. Like *recD* cells, they have normal growth rates.[31] Wild-type Hfr cells are, however, an exception to this rule. In the presence of Gam protein, they grow extremely slowly. We have showed that the effect on their growth results from the interaction between the RecBCD-Gam complex and the integrated F plasmid.[31] Thus, Gam protein is not a "pure" inhibitor of RecBCD enzyme; the RecBCD-Gam complex appears to retain certain catalytic activities. The functional RecD subunit is apparently required for the retention of these activities since Gam protein has no effect on the growth of Hfr cells mutated in the *recD* gene.[31]

Genetic recombination. The presence of Gam protein within wild-type bacteria does not affect recombinational frequencies following Hfr-mediated conjugation and P1-mediated transduction.[21,28] The possibility of other recombination pathways substituting for RecBCD functions may be ruled out.[28] With respect to genetic recombination, Gam protein is thus unable to confer the RecBCD$^-$ phenotype. Interestingly, however, the level of recombination is reduced in the Gam-expressing *recD* mutants.[21] This finding may be taken as additional evidence that the functional RecD subunit is important for the catalytic activities of the RecBCD-Gam complex.

DNA repair. With respect to the effect of Gam protein on DNA repair, the data in the literature have led to conflicting conclusions. Apparently, Gam protein sensitizes the cells to ultraviolet light[21,27] (see also Ref. 32)

whereas it enhances the cellular capacity for repair of X-ray- or gamma-ray-induced DNA lesions.[30,33] The increased resistance to X- or gamma-rays is associated with the improved repair of DNA strand breaks.[30] Efficient rejoining of strand breaks may also explain why the *Eco*K restriction of unmodified λ is dramatically reduced in the presence of Gam protein (Salaj-Šmic, Maršić, Trgovčević and Lloyd, unpublished).

The mechanism of the action of Gam protein is an example of the sophisticated strategy of the phage attack upon the host cell. Despite its modest size (≈16.5 kDa[26]), Gam protein serves at least two functions. By forming the RecBCD-Gam complex, it enables λ to counteract the detrimental effects of RecBCD enzyme. At the same time, the catalytic activities of this complex enable λ to counteract the detrimental effects of the host's *Eco*K restriction system.

The RecD subunit, which is probably essential for the catalytic activities of the RecBCD-Gam complex, contains a consensus adenine nucleotide binding sequence.[34] However, the isolated RecD polypeptide possesses no DNA-dependent ATPase activity.[35] It is likely that this ATPase activity requires a proper interaction between the RecC and RecD subunits (see below). The functions of the RecBCD-Gam complex might depend on the joint action of these two subunits since the third polypeptide, RecB, is bound by the λ-Gam protein.[31]

The RecB polypeptide, which also contains a consensus adenine nucleotide sequence,[36] is critical for DNA unwinding by the RecBCD enzyme.[37] The enzyme mutated in this sequence has no nuclease or ATP hydrolysis activity on double-stranded DNA.[38] In the presence of single-stranded DNA, it, however, retains significant levels of both ATP hydrolysis and nuclease activity. The same might be true for the RecBCD-Gam complex. On the basis of this assumption, we put forward a model for the Gam-dependent repair of DNA strand breaks introduced by the *Eco*K restriction endonuclease.

The *Eco*K restriction endonuclease recognizes specific sequences within the double-stranded DNA (for reviews, see Refs. 39,40). If the recognition sequences are improperly modified, *Eco*K leads to the formation of double-stranded cuts. Each double-stranded cut results from two nicks in the complementary DNA chains and may require two enzyme molecules. Under in vitro conditions, duplex DNA with flush-ended or nearly flush-ended termini is thus generated. These termini bind RecBCD enzyme,[41] which is responsible for acid-solubilization of the restricted DNA within the living cell.[42] However, the action of the *sbcB* gene product, exonuclease I, may precede that of the RecBCD enzyme.[42] Exonuclease I is known to attack duplex DNA molecules with 3' single-stranded tails.[43] We therefore assume that, in contrast to the *in vitro* conditions, DNA termini produced *in vivo* are not necessarily flush-ended. They may possess long 3' single-stranded tails, which are degraded by exonuclease I. When the tails are short enough (≈25 nucleotides), RecBCD enzyme binds DNA and begins its nucleolytic action.

Figure 1A presents the current model for the unwinding and degradation by RecBCD enzyme (*cf.* Ref. 1,5,44). After DNA molecules with short 3' single-stranded tails have been formed (step a1), RecBCD enzyme binds to DNA (step a2) and initiates unwinding. Although the RecB polypeptide is an active helicase (see above), both RecB and RecD are proposed to act as translocating subunits. They, however, translocate along the DNA strands at different rates. In front of the RecB subunit (which moves with a 3' → 5' polarity), a growing single-stranded DNA loop is therefore formed. While un-

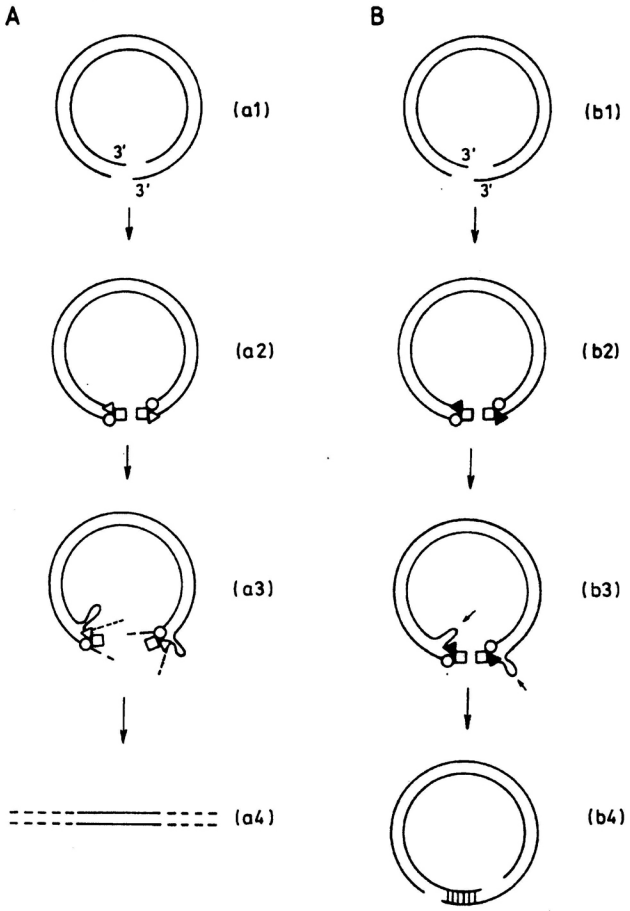

Figure 1. Model of the action of the RecBCD enzyme (A) and the RecBCD-Gam complex (B) on λ DNA that has been cleaved by the *Eco*K restriction enzyme. For details, see text. Symbols: □, the RecC subunit; o, the RecD subunit; Δ, the RecB subunit; ▼, the RecB subunit + Gam protein.

winding DNA, the enzyme degrades both strands. The 3' terminal strand is, however, degraded more vigorously than the complementary strain (step a3). The recombination hot spot Chi (5' G-C-T-G-G-T-G-G 3'),[45] which attenuates the nucleolytic activity,[44] is absent from the DNA of wild-type λ.[46] Therefore, both λ DNA strands are eventually broken down to acid-soluble pieces (step a4).

The catalytic activities of the RecBCD-Gam complex differ from those presented above. In our model (Figure 1B), the RecBCD-Gam complex binds DNA molecules with short 3' tails as does RecBCD enzyme (steps b1 and b2). By hydrolyzing ATP, the RecD subunit is then able to move the whole complex in such a way that a blunt-ended DNA with a loop on the 3' terminal strand is formed. Further movement of the complex as well as DNA unwinding is prevented by Gam protein, which is bound to the RecB polypeptide. The stalled RecBCD-Gam complex can, however, introduce a cut into the loop structure (indicated by the arrow; step b3). A short 3' OH single-stranded DNA tail is thus produced. Its complementary DNA chain is on the other side of a double-stranded break. Homologous recombination system of the phage (the Red pathway) may therefore enable λ DNA to circularize (step b4). Finally, the gaps in DNA are filled and the original structure of λ circles is re-established.

The above model incorporates the unpublished findings that a substantial fraction of the phage can escape restriction in the presence of the RecBCD-Gam complex, Red system and exonuclease I. With slight modifications, the same model might also explain the role of the RecBCD-Gam complex in repair of the host chromosome and in recombination following Hfr conjugation and P1 transduction.

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SAŽETAK

Interakcija bakterije *Escherichia coli* i njezina virusa λ: uloga kompleksa RecBCD-Gam

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Biokemijski su pokusi pokazali da protein Gam iz bakterijskog virusa λ inhibira sve katalitičke aktivnosti enzima RecBCD bakterije *Escherichia coli*. Genetički podaci u nesuglasju su s tim pokusima. Protein Gam, koji se veže s enzimom RecBCD, ima značajan utjecaj na fiziologiju bakterijskog virusa λ i njegova domaćina. Taj se utjecaj ne može objasniti inhibitornom aktivnošću proteina Gam: očito je da kompleks RecBCD-Gam zadržava stanovite enzimske aktivnosti. Predložen je mehanizam kojim te aktivnosti pomažu značajnoj frakciji virusa da izbjegnu restrikciju izazvanu enzimom *Eco*K.