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λp*lac*10 Transducing bacteriophage: DNA primary structure of the region of the abnormal excision

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In studying molecular mechanisms of the formation of transducing bacteriophages, we have elucidated the primary structure of the phage-bacterial DNA junction which resulted from the abnormal excision of the λp*lac*10 phage. The process is structurally similar to the excision of the λp*lac*5 phage and involves, in both cases, highly homological DNA stretches approximately 20 bp long, one of them being a part of the Z-Y spacer of the *lac* operon and possessing a developed secondary structure. The conception of regioselective recombination as a type of illegitimate recombinational process with a certain degree of site-specificity is suggested.

Transducing bacteriophage; Abnormal excision; Phage-bacterial DNA junction; Regioselective recombination (λp*lac*10 phage)

1. INTRODUCTION

Transducing bacteriophages constitute a biologically important group of partners in the exchange of genetic information in prokaryotes; excision of their DNA from the genome of lysogenic bacteria leads to the in vivo formation of recombinant DNA [1]. Although many publications deal with this class of bacteriophages, molecular mechanisms of their formation are almost totally unknown. We have been studying the structural basis of this process as exemplified by λp*lac* transducing bacteriophages constructed in J. Beckwith's group [2,3]. Formation of these phages was largely the result of two independent recombinational events: a long-stretched deletion which joined the proximal end of the *lac* operon with the phage DNA, and the prophage abnormal excision which yielded another junction of both DNAs. Previously, we have established the primary structure of the recombination of the widely known transducing bacteriophage, λp*lac*5, thus having elucidated the total primary structure of its genome [4–7]. The present paper describes structural analysis of the abnormal excision's region in the DNA of another λ*lac* phage, viz. λp*lac*10.

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2. MATERIALS AND METHODS

Restriction endonucleases *Eco*RI, *Mva*I, *Bcn*I and *Sma*I were purchased from Ferment (Vilnyus), *Ava*I and *Nco*I from Vektor (Novosibirsk), DNA polymerase I (Klenow fragment) from Boehringer; *Kpn*I and *Hae*III were isolated by the methods in [5], *Mlu*I was kindly given by A. Solonin (Pushchino) and *Tha*I was given by O. Nekrasova (Moscow). Bacterial strain *Escherichia coli* DP50, supF [F⁻, *ton*A53, *dap*D8, *lac*Y1, *gln*V44 (*sup*E44), Δ(*gal-uvr*B)47, λ⁻, *tyr*T58 (*sup*F58), *gyr*A29, Δ(*thy*A57), *hsd*S3] [8] was used as a host for the λp*lac*10 phage; using a modified technique (phage absorption at 0°C and growth at 39°C in LB medium supplemented with 5 mM $CaCl_2$) [9], we obtained, even from low-titer phage preparations (ca. 10^6 pfu/ml), a rather high yield of the phage (over 1 mg phage DNA from 1 l of culture liquid). Other techniques used in this work were described earlier [5,7].

3. RESULTS

Restriction analysis of λp*lac*10 DNA by means of some rare-cutting restriction endonucleases showed that this DNA, like λp*lac*5 DNA, contains the *Eco*RI site of the *E. coli lac* operon and both *Kpn*I sites of λ DNA, but does not contain the first *Sma*I site of the phage DNA (figs.1 and 2). It means that the phage-bacterial

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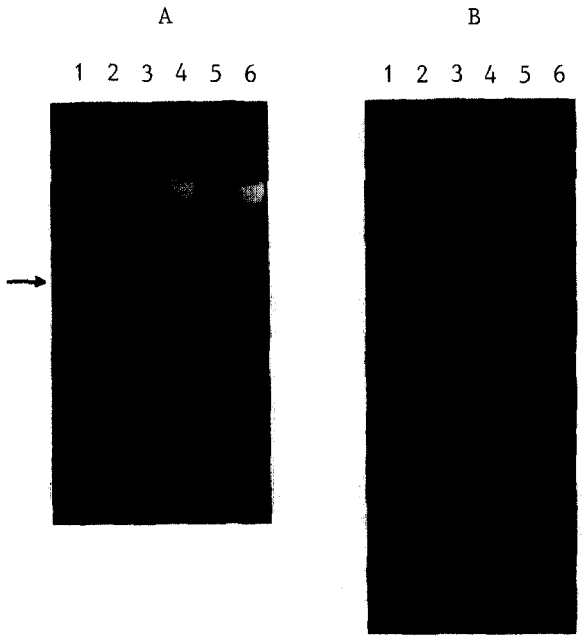

Fig.1. Digestion products of phage DNAs (electrophoresis in 0.7% agarose gel). λ, lanes 1, 4; λp*lac*10, 2, 5; λp*lac*5, 3, 6. (A) *Eco*RI (lanes 1–3), *Nco*I (4–6); the arrow indicates the *Eco*RI fragment of λp*lac*5 and λp*lac*10 DNAs indicating the presence of the *Eco*RI site of the *lacZ* gene. (B) *Mlu*I (lanes 1–3), *Ava*I (4–6); the difference in mobility of the fragments (fifth from above, lanes 2 and 3) illustrates the difference in lengths between *lac*10 and *lac*5 substitutions.

DNA junction in λp*lac*10 is situated within the small *Eco*RI/*Kpn*I segment, ca. 800 bp long. The retained part of the λ genome in λp*lac*10 DNA is shorter than in λp*lac*5 DNA, since the former, in contrast to the latter, lacks the first *Nco*I site of the phage genome (nucleotides 19 329–19 334 according to [10]) (fig.1A, lane 5).

We therefore compared detailed restriction maps of the small *Eco*RI/*Kpn*I fragments of λp*lac*5 [5] and

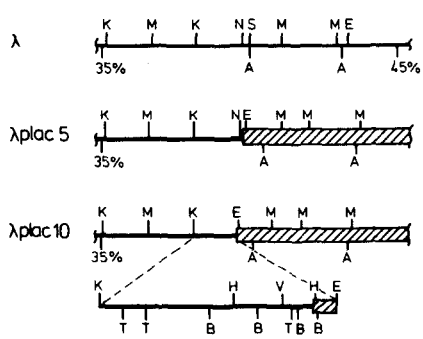

Fig.2. Physical maps of λ, as well as λp*lac*5 and λp*lac*10 DNAs in the region of the phage-bacterial DNA junction which resulted from the abnormal excision of the transducing bacteriophages. A, *Ava*I; B, *Bcn*I; E, *Eco*RI; H, *Hae*III; K, *Kpn*I; M, *Mlu*I; N, *Nco*I; S, *Sma*I; T, *Tha*I; V, *Mva*I.

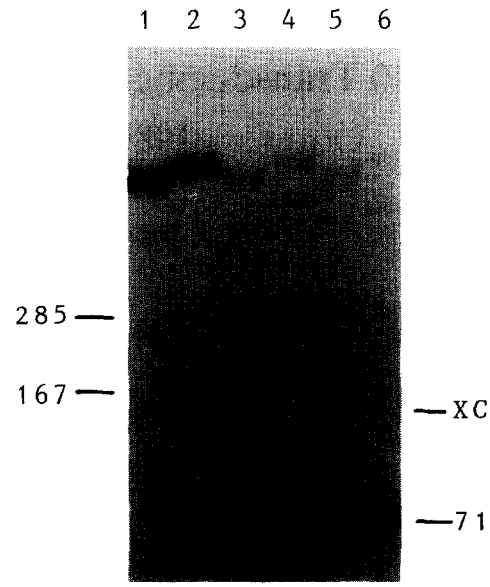

Fig.3. Cleavage of the *Eco*RI/*Kpn*I fragment of λp*lac*10 (800 bp, lane 1) and λp*lac*5 (910 bp, lane 2) DNAs by *Mva*I (lanes 3 and 4, respectively) and *Hae*III (lanes 5 and 6, respectively) (electrophoresis in 10% PAG).

λp*lac*10 DNA (the fragments were labelled at the *Eco*RI termini by means of DNA polymerase I, [α-^{32}P]dATP and dTTP and isolated by electrophoresis in 7% PAG) (fig.3). We found that the phage-bacterial DNA junction which resulted from the λp*lac*10 abnormal excision was located within the *Eco*RI/*Mva*I fragment (167 bp) of λp*lac*10 DNA. Sequencing this fragment by the Maxam-Gilbert method enabled us to identify the DNA segments in the *E. coli* and λ genomes involved in the λp*lac*10's abnormal excision process and corresponding to the crossover region (fig.4).

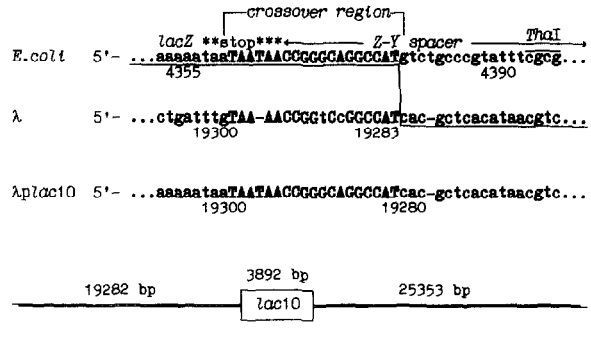

Fig.4. Primary structures of the recombining partners (λ [10] and *E. coli* [11] DNAs) and the product of the excisional recombination (λp*lac*10 DNA) in the region of the phage-bacterial junction, and the scheme of the λp*lac*10 genome (48 527 bp, our data).

4. DISCUSSION

Previously we found that abnormal excision of the λp*lac*5 transducing bacteriophage involved structurally homological areas ca. 20 bp long (17 identical nucleotides out of 21, 81% homology) [4,5]. As fig.4 shows, λp*lac*10 excisional recombination occurred along similar lines: in the recombination areas of both partners (λ and *E. coli* DNA), 16 out of 19 nucleotides coincide, accounting for 84% homology. In both cases (λp*lac*10 and λp*lac*5 formation), the same loci take part in the excisional recombination, viz. the *lom* gene from λ DNA and the Z-Y spacer of the *lac* operon (*E. coli* DNA). The homology-dependence of the two excisions is illustrated by the comparative computer analysis of the interacting segments (*lom* gene and Z-Y spacer), showing that optimal, in terms of homology, segments were chosen (the homology matrices are not presented).

It should be noted that the similarity of both excisional recombinations goes even further and involves some higher structure features. Earlier we suggested a hairpin structure in the intergenic Z-Y segment as an intermediate of the λp*lac*5 excisional recombination [5]; the free energy of this hairpin's formation is −14.6 kcal/mol. The abnormal excision of λp*lac*10 appears to also occur within that hairpin, even if not in its single-stranded part. At the same time, the Z-Y spacer can be presented in terms not only of the above hairpin, but alternatively, in terms of two adjacent hairpins of smaller sizes (fig.5). It is noteworthy that in this version of the secondary structure both crossover points (in λp*lac*5 and λp*lac*10 excisions) are located in the apical parts of the loops within the octanucleotide AATC-CATT and hexanucleotide GGCCAT, respectively, containing a common sequence of CCAT (fig.5).

It thus follows that excisions of transducing bacteriophages λp*lac*5 and λp*lac*10 resemble each other in both structural features and possible recombinational intermediates. We consider the bacterial partner, i.e. the intergenic Z-Y segment, belonging to non-coding DNA sequences whose higher structure and role in the function of bacterial operons are still unknown, as a 'donor' component of the excisional recombination. It is within this segment that prerequisites of the recombinational event may arise, viz. appropriate hairpin (cross-like) structures exemplified by the previously described model for λp*lac*5 [5] or by the above rather stable hairpin ($\Delta G = -22.2$ kcal/mol) for λp*lac*10 (fig.5). In the course of the further development of the recombinational event, these transient structural elements of the genome might function as a sort of probe, searching, in a regioselective manner, the second partner's DNA for segments appropriate (in terms of structural homology) for recombination. The regioselectivity is thought to be largely determined by DNA homology, i.e. resemblance of the nucleotide sequences ca. 20 bp long. The driving force of the recombination

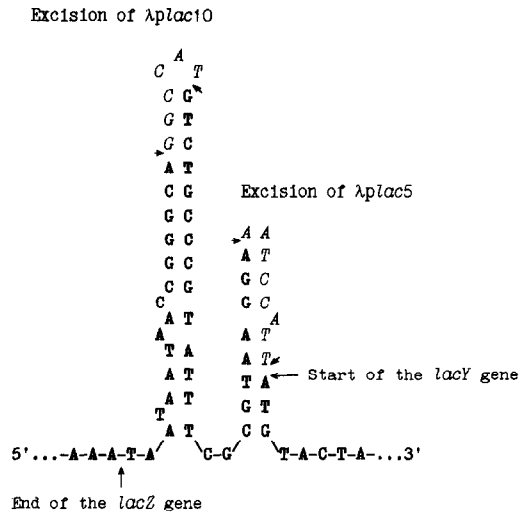

Fig.5. The putative secondary structure of the intergenic Z-Y segment of the *lac* operon (a hypothetical intermediate in excision of λp*lac*10 and λp*lac*5 phages).

may be enzyme(s) recognizing apical parts of hairpins (or similar elements of the DNA secondary structure); a possible additional factor providing regioselectivity is the enzyme(s)'s sequence specificity, e.g. towards sequence CCAT or complementary ATGG (this, by the way, is an analogue of tetranucleotide GTGG, whose possible role in prokaryotic illegal recombination was discussed previously [7,12]).

Finally, an evolutionary implication of the regioselectivity of some illegitimate recombinations should be emphasized. We suppose that this is a relatively simple way through which more subtle and sophisticated mechanisms of site-specific genomic rearrangements might be developed. Not only secondary structures may be considered as intermediates of regioselective recombination (this being the simplest case), but also evolutionary more elaborated nucleoproteins carrying out the capture of the second (acceptor) recombination partner. This way might already be very close to site-specific recombination (cf. e.g. the mechanism of site-specific integration of the λ phage into the *E. coli* chromosome [13]).

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