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*MULTIPLE SITES OF INTERACTION WITH HOST-CELL DNA IN THE DNA OF PHAGE λ**

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During lysogenization of *Escherichia coli* by phage λ, the DNA of the phage is incorporated into that of its host to form a stable, compound chromosome. The mechanism of incorporation is unknown, but presumably calls for interactions at sites of complementary base sequence in the two DNA's. The existence of such sites of interaction was demonstrated by Cowie and McCarthy[1] and by Green.[2]

The isolation of specific parts of the λ DNA molecule[3, 4] makes possible attempts to determine the intramolecular positions of the reactive sites. In principle, genetic deletions can be utilized for the same purpose. Preliminary experiments along both lines are reported in this paper.

Materials and Methods.—Bacterial DNA was prepared from *E. coli* strain B (Berkeley) or W3110 (sensitive to phage λ) by the method of Marmur.[5] DNA from phage λ, genotype cb^+ unless otherwise stated, labeled with P^{32} at 1–10 mc/mg P, was prepared according to Burgi.[6] Interaction of the two DNA's was measured by the DNA-agar method of Bolton and McCarthy.[7] The solvent used, known as standard saline-citrate (SSC), contains 0.15 *M* NaCl and 0.015 *M* sodium citrate. The terms 2 × SSC, SSC/30, and SSC/100 signify multiple or fractional concentrations of both salts.

Molecular ends of λ DNA were prepared as follows.[4] P^{32}-labeled DNA was reduced to approximate quarter-length fragments by mechanical stirring. The two terminal quarters were then rejoined through their cohesive sites by heating the solution (in 0.6 *M* NaCl) briefly to 75°C and cooling it slowly.[8] The joined fragments were separated from the others by subjecting the mixture to preparative centrifugation through a density gradient of sucrose and recovering the faster-sedimenting band. The isolated terminal fragments were disjoined by heating the

solution to 75°C (in 0.1 *M* NaCl) and cooling it quickly. The mixture was next passed through a methylated serum albumin column operated at 4°C.[3] The fractions eluting first under a concentration gradient of NaCl contained "left" ends. Fractions eluting last contained "right" ends but were contaminated with left ends. Pooled fractions of either kind were purified further by a repetition of the thermal-joining and centrifugation steps, this time recovering fragments that failed to join, representing mainly the excess over numerical equality of left or right ends, respectively. Left and right ends are so called with reference to the genetic map,[3] and are recognized physically by the fact that left ends contain more guanine and cytosine, and are consequently denser, than right ends.

Molecular weights[9] of the isolated materials varied between 0.26 and 0.32 of that of intact λ DNA (31×10^6). Owing to fractionation with respect to size in the methylated albumin column, the recovered left ends were somewhat shorter than the right ends. Purity, as judged by density analysis, was about 90 per cent for right ends, and higher for left ends, as shown in Figure 1.

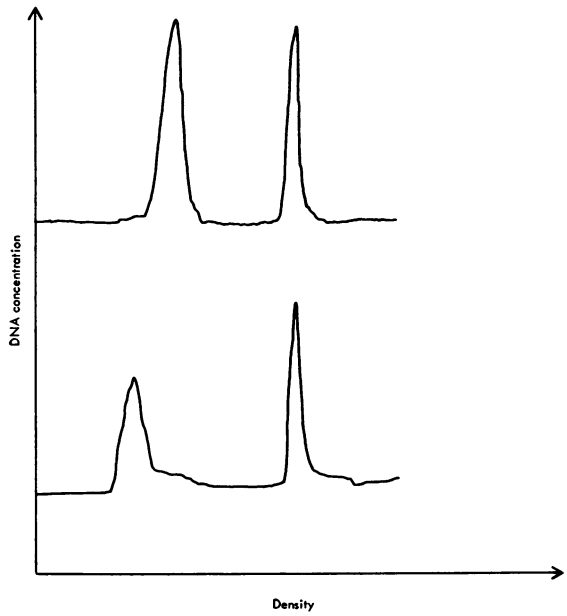

FIG. 1.—Bands formed at equilibrium in a density gradient of CsCl. Left ends of λ DNA molecules (density 1.715, upper diagram) and right ends (density 1.706, lower diagram), each representing about 28% of the original molecular length. Reference bands at the right are *Bacillus subtilis* phage SP8 DNA of density 1.742. λ DNA preparations were heated to 75°C for 1 min, cooled in ice, and spun in CsCl at 5°C to prevent spontaneous joining of left- or right-end contaminants to the major fractions. Photographed after 30 hr at 44,770 rpm.

Agar containing denatured *E. coli* DNA was prepared according to Bolton and McCarthy.[7] P^{32}-labeled λ DNA, either unfractionated or the isolated molecular ends, was reduced to fragments of about 400,000 daltons by passage through a French pressure cell or by sonic treatment. The fragments were denatured by heating in 2 × SSC, and mixed with DNA agar. The mixture was heated in sealed

tubes at 60°C for 18–24 hr to permit specific interactions to occur, and transferred to water-jacketed columns for analysis.

The b^+ region of lambda DNA, representing base sequences present in wild-type λ but missing in the deletion mutant λb_2, was isolated by absorbing out cross-reacting material from P^{32}-labeled λcb^+ DNA fragments on λcb_2 DNA agar. The fraction failing to attach in two or more treatments contained 20–26 per cent of the total labeled DNA. It was concentrated by evaporation, dialyzed against 2 × SSC, and centrifuged to remove precipitated agar.

Results.—Left and right molecular ends: The ability of λ DNA and its molecular ends to form specific complexes with *E. coli* DNA is illustrated in Table 1. Comparing results presented in the individual rows, one sees that left ends, right ends, and unfractionated λ DNA react about equally. Comparing vertical columns, one sees that the fraction of λ DNA bound depends on the absolute and relative concentrations of the two DNA's (as well as other variables) in the mixture. The strong dependence on the concentration of *E. coli* DNA in the agar is understandable, since only 0.2 per cent of that DNA participates in the reaction.[1] The fraction of λ DNA participating is about 34 per cent.[1]

TABLE 1

SPECIFIC ATTACHMENT OF λ DNA FRAGMENTS DERIVED FROM DIFFERENT PARTS OF THE MOLECULE TO *Escherichia coli* DNA AGAR

E. coli DNA in agar (μg)	Labeled λ DNA (μg)	Labeled DNA Bound (%)		
		λ Unfractionated	λ Left ends	λ Left ends
20 (W3110)	0.02	12.7	8.3	8.0
220 (W3110)	0.04	23.3	18.9	—
180 (BB)	0.005	27.6	24.7	19.3
12 (BB)	0.005	17.9	13.0	16.4
24 (BB)	0.005	18.1	12.1	17.4

The mixtures contained 0.2 gm of DNA-agar and an equal volume of labeled DNA in 2 × SSC, and were held for about 18 hr at 60°C, transferred to a column, and washed with ten 10-ml portions of 2 × SSC at 60°C. The specifically bound, labeled DNA remaining in the agar was removed for assay by washing with SSC/100 at 75°C.

The ability of the central portion of the λ DNA molecule to react with *E. coli* DNA can be estimated by difference from the data of Table 1. Such estimates suggest that the reactive sites may be appreciably more concentrated near the center of the molecule than near its ends. However, the measurements are biased by the fact that, at equal DNA concentrations, the concentration of reactive fragments specified with respect to kind is higher in the fractionated than in the unfractionated preparations. The only safe conclusion is that both terminal and central parts of the λ DNA molecule contain reactive sites.

The left end of the λ DNA molecule contains about 56 per cent guanine and cytosine, and the right end about 47 per cent (Fig. 1 and unpublished data). The melting temperatures of the two ends reflect the difference in composition. If the sequences interacting with *E. coli* DNA differ in composition in the same way as the molecular parts in which they lie, one might expect fragmented left ends to melt out of *E. coli* DNA agar at a higher temperature than right ends.

Thermal chromatograms presented in Figure 2 show in fact that the bonds by which the left ends of λ DNA attach to *E. coli* DNA are more stable to heat than those involved at the right end. Both ends, however, show high- and low-melting components. The chromatogram for left ends exhibits maxima at 60, 66, and 72°C,

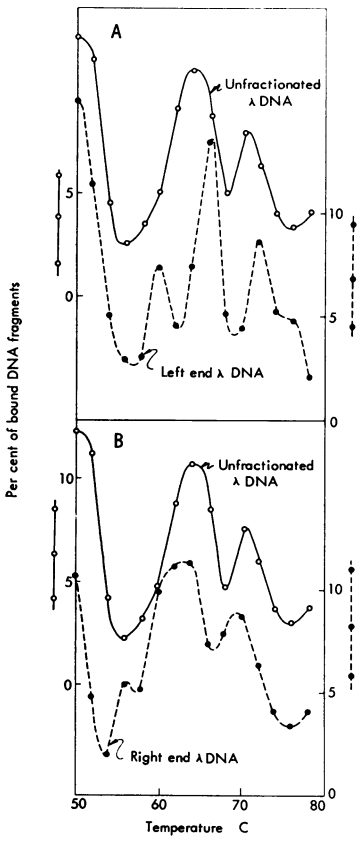

FIG. 2.—Thermal chromatograms of complexes formed between *E. coli* DNA and different parts of the lambda DNA molecule. The mixed DNA's were annealed at 60°C in 2 × SSC in the usual way, transferred to a column, and washed with ten 10-ml portions of 2 × SSC followed by two portions of SSC/30 at 50°, each portion passing through the column in about 10 min. Thereafter, the DNA removed in a single wash with SSC/30 at each of a series of temperatures was measured. The results presented in the figure were obtained with agar containing about 100 μg DNA/gm. When agar containing DNA at higher concentrations was used, much of the labeled DNA eluted at lower temperatures and characteristic patterns were not visible.

that for right ends at 63 and 69°C (in SSC/30). The chromatogram for unfractionated λ DNA is roughly a composite of the two extremes. It seems likely, therefore, that even within individual sections of the molecule there are multiple segments capable of interaction with *E. coli* DNA.

The b_2^+ region: Another method of isolating specific parts of the DNA molecule depends on the use of genetic deletions. The b_2 deletion mutant proved suitable for this purpose. The mutant phage contains a DNA molecule about 18 per cent shorter than that found in wild-type λ.[6, 10] The site of the deletion has not been determined directly, but the loss is believed to have occurred near the host-range (*h*) locus, to the left of the molecular center, in a region important for lysogeny.[11] It is probably a region rich in adenine and thymine, since the mutant DNA contains a diminished proportion of these bases.[12, 13]

Isolation of the b_2^+-specific region of the λ DNA molecule is described in the *Materials and Methods* section of this paper. Its interaction with *E. coli* DNA is shown in Table 2. Since the material was not highly purified, competing unlabeled b_2 DNA was used to achieve the desired specificity. The results show that the b_2^+ region of the λ DNA molecule, like the other regions tested, contains sites capable of reacting with *E. coli* DNA. Green[2] and Kiger and Green[14] made the complementary test and found that λb_2 DNA contains most of the sites reactive with *E. coli* DNA present in λ wild-type DNA. Their results left open the possibility that the b_2^+ region contains no sequences in common with *E. coli*.

The specificity of interaction: The specificity of interactions of the type studied here has been checked repeatedly by Bolton and McCarthy and others. We found, in addition, that labeled λ DNA fragments do not bind to T5 DNA agar. The finding of Cowie and McCarthy[1] that the interacting segments in the DNA's of λ and *E. coli* are of about the same length signifies that there are unique complementary sequences present in the two DNA's. This conclusion was checked by applying labeled *E. coli* DNA fragments to λ DNA agar, which showed no measurable attachment (theoretical expectation about 0.2%) in excess of the small background seen with agar containing no DNA. Finally, the high melting temperature of the complexes formed by labeled λ fragments in *E. coli*

TABLE 2

SPECIFIC ATTACHMENT TO DNA AGAR OF NUCLEOTIDE SEQUENCES (λb^+ SEGMENT) PRESENT IN λ WILD-TYPE BUT NOT IN λb_2 DNA

Labeled DNA	DNA in Agar		
	λb^+	λb_2	*E. coli* W3110
Unfractionated λ	79	73	13
λb^+ segment	72	26	9
λb^+ segment*	55	11	6

Each test mixture contained 0.2 gm agar (10–20 μg DNA) + 0.002 μg P^{32}-labeled DNA, sheared and denatured.
The numbers express % of labeled DNA not eluted by 2 × SSC at 60°C.
* Each test mixture contained in addition 1 μg (5 μg in other experiments) of unlabeled, sheared, and denatured λb_2 DNA as competitor.

DNA agar (Fig. 2), similar to that found for λ-λ DNA complexes, signifies authentic matching of numerous base pairs. Kiger and Green[14] reached the same conclusion after studying the thermal stability of the appropriate DNA-RNA complexes.

Discussion and Conclusion.—The homology test of Bolton and McCarthy as employed in this work consists in reducing lambda DNA molecules to about 150 single-stranded fragments and determining what fraction of them can attach to denatured *E. coli* DNA.

The previous experiments[1] had shown that about one third of them can attach under optimal conditions. The present results show that the 50 or so reactive fragments are derived more or less equally from the left, right, and central quarters of the molecular length. In addition, the 18 per cent stretch in λ wild-type DNA corresponding to the b_2 deletion yields reactive fragments. Finally, analysis in terms of melting temperature of the complexes further subdivides the fragments derived from any one section of the molecule into two or three subclasses. The inference is clear that there are at least three and probably more sites on the λ DNA molecule that can interact with *E. coli* DNA. However, the actual number, length, and distribution of these sites remains to be determined.

The results described do not throw much light on questions concerning the origin and function of the homology between λ DNA and the DNA of its host, nor about the role of the common sequences in lysogeny. One possibility can be excluded, however. The stretches involved cannot belong to a class of periodically repeated sequences present in both DNA's, because in the DNA of *E. coli* they are relatively very rare. It should prove instructive, therefore, to ascertain the location of the cross-reacting sites in the genome of *E. coli*.

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*PLEIOTROPIC EFFECTS OF SUPPRESSORS OF A LAC-"OPERATOR NEGATIVE" MUTATION IN ESCHERICHIA COLI**

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A suppressor mutation is a genetic change which removes the mutant phenotype associated with another mutation, and which is separable from the first mutation by recombination. Of particular interest are suppressor mutations which act at the level of translation of the gene modified by the first mutation. In this context, it is useful to consider if the lesion occasioned by the original mutation leads to the formation of a mis-sense or nonsense triplet. A mis-sense triplet results in an amino acid substitution,[1] while a nonsense triplet results in an untranslatable codon.[2] Extragenic suppression of a mis-sense triplet has been demonstrated for an allele of the tryptophan synthetase system[3] of *Escherichia coli*. In the same organism, extragenic suppression of a nonsense triplet has been demonstrated for certain *ambivalent* alleles of the A cistron of the *r*II system of phage T4,[1] for certain *amber* mutants of the cistron which codes for the head protein of T4,[4] for a mutant (O_2^0) allele of the β-galactosidase structural gene,[5, 6] and for a mutant allele of the alkaline phosphatase structural gene.[7]

The mechanism(s) of suppression of mis-sense or nonsense triplets has not been demonstrated experimentally. It has been speculated that alterations of either an activating enzyme or a transfer-RNA could account for suppression.[8] More recently, ribosomes have been implicated as another component whose alteration can result in suppression.[9] Needless to say, alteration of any component of the translation system which does or can interact with messenger-RNA during the translation process might result in suppression. Whatever the mechanism, it might be expected that alteration of the translation machinery of a cell would, in certain instances, affect the translation of genetic information other than the original mutant triplet.[3] This type of alteration of the translation machinery could be indicated by an unusual physiological behavior of a strain harboring the responsible suppressor mutation.

In this paper, we report the properties of certain mutants capable of suppressing the *lac*$^-$ phenotype determined by the "operator negative" mutation[10] in strain 2320(λ) of *E. coli*. The suppressors were selected for further study because of their concomitant acquisition of (*a*) the ability to suppress a set of *r*II mutants of phage T4 and certain mutants of T7,[6, 11] and (*b*) a variety of new physiological properties not obviously related to suppression of the *lac*$^-$ phenotype. Suppression of the *lac*$^-$ phenotype is thought to be accomplished by the acquisition of the ability to translate the nonsense triplet in the O_2^0 allele in strain 2320(λ).[6, 12] Pleiotropic ef-