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PROGRESS TOWARD A METABOLIC INTERPRETATION OF GENETIC RECOMBINATION OF *ESCHERICHIA COLI* AND BACTERIOPHAGE LAMBDA

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DNA is a metabolically active cellular component of bacteria. Transcription and replication can proceed continuously throughout the growth and division of bacterial cells. Enzymes methylate DNA bases and when they have not acted restriction enzymes nick and break double-stranded DNA molecules producing substrates for further degradation. When radiations or chemicals damage DNA, repair enzymes either change or remove damaged portions, restoring the DNA to its original state, or they deal with secondary lesions produced by faulty metabolism of the unremoved primary lesions.

In addition to these realms of DNA metabolism there is the realm of genetic recombination. A metabolic interpretation of genetic recombination will require knowledge of the structures of the starting materials and end products of recombination. It will also require a list of the enzymes or proteins catalyzing the steps in recombination together with their activities and substrate specificities and ultimately the intermediates and products of each step. Furthermore a metabolic interpretation will require a knowledge of the regulation of the synthesis and activities of the enzymes and other proteins of recombination. Finally the relationships between recombination and the other realms of DNA metabolism must be stated as a list of common and unique intermediates and enzymes.

At present we are far from such a metabolic interpretation although considerable progress has been made. In the present report I shall emphasize work performed in my own laboratory on the recombination of *E. coli* and bacteriophage lambda. Several recent reviews (e.g., RADDING 1973; CLARK 1973; HOTCHKISS 1971; SIGNER 1971; WHITEHOUSE 1970) show this work to be but a small part of the total effort on recombination. These reviews should be consulted to obtain a truly accurate view of the progress toward a metabolic interpretation.

In my own laboratory I and my colleagues have concentrated on obtaining three types of information: (1) a list of enzymes involved in recombination of *E. coli* and their properties; (2) a description of the recombination behavior of strains carrying mutations affecting these enzymes and the one or more pathways of recombination in which they act; and (3) a description of the relationships existing between the pathways of recombination of bacteriophage lambda and those of *E. coli*. The inclusion of lambda in our considerations of bacterial recombination has many advantages not the least of which is the large amount

TABLE 1

Properties of four enzymes associated with recombination of E. coli *and bacteriophage lambda**

	ExoV	ExoI	ExoVIII	Exoλ
Structural gene(s)	*recB, recC*	*sbcB*	*recE*	*redX*
Molecular weight	2.7×10^5d	5.5×10^4d	ca 1.3×10^5d (subunit)	2.4×10^4d
Exonuclease activity†				
Native DNA	2 to ½	1	50	350
Denatured DNA	1	40,000	1	1
Direction of action				
Native DNA	5′→3′ ; 3′→5′	n.a.	unknown	5′→3′
Denatured DNA	5′→3′ ; 3′→5′	3′→5′	unknown	unknown
Endonuclease activity†				
Native DNA	1	—	unknown	—
Denatured DNA	10,000	—	—	—
Dependence of activity on ATP	+ (exo) ± (endo)	—	—	—
Action initiated at Nicks	—	n.a.	unknown	—

* Properties of ExoV were obtained from GOLDMARK and LINN (1972). Properties of ExoI were obtained from LEHMAN (1960) and from LEHMAN and NUSSBAUM (1964). Properties of and procedures for purifying ExoVIII will be published in a paper by S. R. KUSHNER, H. NAGAISHI and A. J. CLARK. Properties of Exoλ were obtained from LITTLE (1967) and from CARTER and RADDING (1971).

n.a. indicates that the subject is not applicable to the enyme in question. Minus sign indicate no detectable activity is reported.

† Activity on one substrate taken to be unity; other activity is relative to the first.

of current information on lambda recombination and replication which is being obtained in several laboratories (e.g., see HERSHEY 1971). In addition, the first enzyme definitely associated with recombination is one determined by the *redX* gene of lambda, the lambda exonuclease or Exoλ (SIGNER *et al.* 1968). The properties of Exoλ are listed in Table 1. Most salient for this discussion are the facts that this is a small enzyme which is much more active on native than on denatured DNA and that it operates in the 5′ to 3′ direction preferring 5′-phosphoryl terminated strands (LITTLE 1967). As a result of this set of properties a limit digest of native DNA contains about half the original substrate as high molecular weight single strands and about half as mononucleotides (LITTLE 1967). Another important property of Exoλ is that it binds to but does not initiate digestion from nicks in duplex DNA (CARTER and RADDING 1971). This property together with those mentioned previously have inspired suggestions for several ways in which Exoλ may act to catalyze lambda recombination in particular and recombination of bacteria and fungi in general (CASSUTO and RADDING 1971; RADDING 1973; MESELSON and RADDING, personal communication).

From *E. coli* a nuclease connected with recombination has now also been characterized (BUTTIN and WRIGHT 1968; OISHI 1969; BARBOUR and CLARK 1970). It is called exonuclease V (i.e. ExoV) and is determined by the *recB* and *recC* genes. This enzyme, by contrast to Exoλ, is large and is composed of two

nonidentical subunits of nearly the same molecular weights (GOLDMARK and LINN 1972). ExoV acts on native DNA but it does so at rates close to those on which it acts on denatured DNA. The variable relative rates are determined by the concentration of ATP, a cofactor which is required for both exonuclease activities of ExoV yet which inhibits the activity on native DNA at high concentrations (GOLDMARK and LINN 1972). The limit digest of ExoV action on native DNA consists entirely of acid soluble products (i.e., oligonucleotides and mononucleotides) consistent with its ability to degrade both native and denatured DNA (GOLDMARK and LINN 1972; TANNER, NOBREGA and OISHI 1972). In this regard it also differs from Exoλ. Finally ExoV has endonuclease activity on single-stranded DNA and a DNA dependent ATPase activity both not possessed by Exoλ (GOLDMARK and LINN 1970).

Clues to the functions of this complex enzyme are provided by examination of mutants of the *recB* or *recC genes* (see CLARK 1973 and RADDING 1973 for specific references). In absolute mutants all activities of ExoV are absence. In addition the following effects are noted: (1) conjugational and transductional recombinant frequencies are reduced to between 0.3% and 20% of wild type values; (2) recovery of cells from UV and X irradiation and mitomycin C treatment is reduced; and (3) nondividing cells and cells capable of only a small number of divisions occur at appreciable frequencies. Attempts to interrelate these effects theoretically ran into the difficulty that it was unclear which of the activities of exonuclease V were involved in recombinational, repair and replicational metabolism. Recent experiments performed by S. KUSHNER (personal communication) have yield the first evidence that the exonuclease activity of native DNA is involved both in recombinational and repair metabolism. A mutant carrying *recB270* and *recC271* was used for these experiments. This mutant strain was originally described by TOMIZAWA and OGAWA (1972). At 30° this strain is somewhat Rec$^-$ and somewhat UV^s compared with a wild-type strain. At 43° it becomes more Rec$^-$ and more UV^s; thus it acts like a temperature-sensitive mutant strain. From this strain DR. KUSHNER purified exonuclease V. He found all four activities of this purified enzyme to be present in cells grown at 30° at reduced specific activities compared with the wild type. Of the four activities only the double-stranded exonuclease was temperature-sensitive. From these results DR. KUSHNER concludes that at least the double-stranded exonuclease activity of ExoV is important for both recombination and repair.

At the present time it also appears that the double-stranded exonuclease activity may be involved in replicational metabolism since a culture of cells carrying *recB270 recC271* grown at 43° contains a higher proportion of inviable cells than a culture grown at 30° (KUSHNER, personal communication).

The synthetic abilities of nondividing inviable cells found in a culture of a *recB21* and *recC22* mutant have been investigated by F. CAPALDO and S. D. BARBOUR (personal communication). These cells do not perform vegetative chromosome replication as evidenced by their inability to incorporate radioactive thymidine. Their capacity to carry out repair synthesis or conjugational transfer synthesis remains to be determined, but it would not be surprising to those in

my laboratory and DAVID ZUSMAN's laboratory to learn from CAPALDO and BARBOUR at some future time that it is initiation of vegetative chromosome replication and not the capacity for DNA synthesis which is irreversibly blocked in these nondividing inviable cells.

One of the most intriguing metabolic features of the absence of exonuclease V is the ability of different strains of *E. coli* to compensate for this absence indirectly either by inactivating a constitutive DNA nuclease (KUSHNER, NAGAISHI and CLARK, 1972) or by expressing an otherwise unexpressed DNA nuclease (BARBOUR and CLARK 1970). In the first case mutations called *sbcB*$^-$ occur in what is probably the structural gene of exonuclease I (i.e., ExoI). In the second case mutations called *sbcA*$^-$ occur in what may be a regulator gene derepressing a DNA nuclease we have called exonuclease VIII (i.e., ExoVIII). In each case the recombination deficiency, repair deficiency, and inviability of exonuclease V deficient strains essentially disappears and the wild-type phenotypes are restored (CAPALDO-KIMBALL and BARBOUR, 1971; TEMPLIN and CLARK, unpublished). Drawing upon the concept that recombination is one type of DNA metabolism BARBOUR and CLARK (1970) and KUSHNER, NAGAISHI and CLARK (1972) hypothesized that the indirect suppression activity of *sbcB* and *sbcA* mutations occurs by facilitating or opening up pathways of recombination alternative to the one involving ExoV and the *recB* and *recC* genes.

The hypothesis that ExoI interferes with full expression of a pathway of recombination alternative to the RecBC pathway has been treated extensively in two other articles (HORII and CLARK, 1973; CLARK, 1973). At this time the principle evidence for this hypothesis stems from the properties of several mutations which block recombination of a *recB*$^-$ *recC*$^-$ *sbcB*$^-$ strain but do not block recombination in a *recB*$^+$ *recC*$^+$*sbcB*$^+$ or *sbcB*$^-$ strain. Three of these mutations fall in the *recF* gene and the pathway which they affect has been called the RecF pathway. A hypothetical relationship between the RecF and RecBC pathways is shown in Figure 1. In this *sbcB*$^+$ is shown determining the conversion of an intermediate in the RecF pathway into an intermediate in the RecBC pathway. Presumably this conversion is performed by ExoI whose sole activity seems to be the digestion of denatured DNA to mononucleotides in the 3′ to 5′ direction (Table 1). ExoI appears therefore to act indirectly on recombination by the RecF and RecBC pathways. Further explanation of the RecF pathway hypothesis is to be found in the articles previously cited (HORII and CLARK 1973; CLARK 1973).

At this time I would like to examine the current evidence that ExoVIII participates in a pathway alternative to the RecBC pathway. Since Exo VIII may act directly in recombination it seems appropriate to compare its properties with those of ExoV and Exoλ. Exo VIII has been purified by H. NAGAISHI in my laboratory with the collaboration of S. KUSHNER. A detailed report on this work is in preparation (S. R. KUSHNER, H. NAGAISHI and A. J. CLARK, in preparation). In Table 1 is a summary of our results. The enzyme is large with a subunit molecular weight of about 1.2–1.3 $\times$ 10^5d as determined by SDS acrylamide gel electrophoresis of a preparation about 90% pure. Preliminary evidence from gels run on native protein indicates that the full enzyme may be composed of

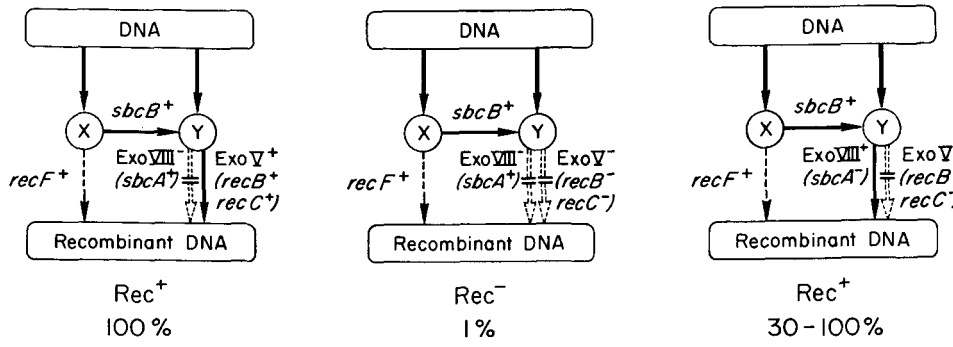

FIGURE 1.—Diagrammatic representation of hypothetical recombination pathways based on the idea that ExoV and ExoVIII may be isozymes. Each frame represents the pathways available for recombination in a strain of the given genotype. Thick solid arrows indicate an indefinite number of functioning steps in recombination. Thin dashed arrows indicate an indefinite number of steps in recombination functioning at lower than maximal effectiveness. Doubled dashed arrows indicate an indefinite number of steps in recombination which are not functioning because of a genetic block somewhere in their sequence. The recombination phenotype is indicated along with the approximate percent conjugational recombinants formed normalized to the wild-type values as 100%.

more than one subunit. In size therefore ExoVIII resembles ExoV rather than Exoλ. By contrast in some of its other properties ExoVIII resembles Exoλ; for example ExoVIII is not dependent on ATP for its activity and it has no endonucleolytic activity on denatured DNA. ExoVIII is much more active exonucleolytically on native than on denatured DNA, a property also similar to that of Exoλ. The crucial points are undetermined at present, however. We have not yet obtained enough pure ExoVIII to determine the nature of its limit digest products. Only after additional work will it be possible to determine the direction of exonucleolytic action of the enzyme and its end group specificity. Thus we can not at present conclude from biochemical data that ExoVIII is an isozyme of ExoV, an isozyme of Exoλ, or a nuclease with different exonucleolytic properties from either of those recombination enzymes.

It is possible to obtain genetic as well as biochemical insight into the action of ExoVIII. To do this I would like to discuss some data obtained by JANE GILLEN in my laboratory (Table 2). What Ms. GILLEN has done is to test the effects of the *recF143* mutation on conjugational recombination in an ExoVIII$^+$ ExoV$^-$ ExoI$^+$ strain. First consider the possibility that ExoVIII is an isozyme of ExoV (Figure 1). In the first panel the RecBC pathway is shown operating in the presence of ExoV and the absence of ExoVIII consistent with a *recB*$^+$ *recC*$^+$*sbcA*$^+$ genotype. In the second panel mutations in *recB* and *recC* block the RecBC pathway. According to this hypothesis the RecF pathway should be acting ineffectively in recombination because of the presence of ExoI; hence the *recB*$^-$ *recC*$^-$ *sbcA*$^+$ strain is Rec$^-$. In the third frame the RecBC pathway is operating effectively despite the absence of ExoV because of ExoVIII is present and is assumed to be isozyme of ExoV. This is the hypothetical situation in a *recB*$^-$ *recC*$^-$ *sbcA*$^-$ strain. According to this hypothesis a mutation in *recF* would not appreciably

TABLE 2

Dependence upon recA *and* recF *of bacterial and lambda recombinant frequencies in different genetic backgrounds*

Exonucleases* ExoV	ExoVIII	ExoI	Inferred Rec pathways	Genotype *recA*	*recF*	Recombinant frequencies (%)† Hfr TL+[SmR]	Lambda (A to J) *red+gam+*	*red-gam-*
				+	+	6	3.0	2.0
+	—	+	RecBC (mostly	—	+	8×10^{-4}	2.2	0.03
				+	—	6	4.5	2.8
				+	+	4	4.9	3.3
—	+	+	RecE (mostly)	—	+	$<6\times10^{-5}$	4.5	2.9
				+	—	6×10^{-2}	3.6	2.6
				+	+	4	4.8	0.63
—	—	—	RecF	—	+	1×10^{-5}	3.7	0.03
				+	—	6×10^{-4}	2.5	0.01
				+	+	3×10^{-2}	4.5	0.29
—	—	+	None (essentially)	—	+	3×10^{-5}	3.8	0.02
				+	—	3×10^{-4}	3.3	0.08

* To achieve the absence of ExoV *recB21* and *recC22* mutations were used. To achieve the presence of ExoVIII *sbcA23* was used. To achieve the absence of ExoI *sbcB15* was used.

† Bacterial recombinants were produced in 60-minute interrupting matings using a Hayes Hfr strain JC158 as donor. Threonine- and leucine-independent streptomycin-resistant progeny were selected. *susA*$^-$ *susJ*+ and *susA*+ *susJ*$^-$ strains of wild-type lambda or λ*bio10* were crossed and *sus*+ recombinants detected by standard methods (UNGER, ECHOLS and CLARK 1972). Experimental results, except for those in parentheses, are averages of two or more experiments.

affect the recombination proficiency of the strain since *recF* does not act in the RecBC pathway. Ms. GILLEN has found however that conjugational recombinant formation is reduced to approximately 1% of *recF*+ levels by *recF143* in the *recB*$^-$ *recC*$^-$ *sbsA*$^-$ *strain* (Table 2).

The result indicates that ExoVIII cannot be completely isozymic to ExoV. We have consequently formulated the hypothesis diagrammed in Figure 2. Verbally expressed this hypothesis is that ExoVIII acts in a pathway of recombination partially identical to the RecF pathway. For discussion we call this pathway the RecE pathway to indicate the involvement of *recE*, the structural gene of ExoVIII. In Figure 2 both the RecE and the RecF pathways contain a common intermediate which is the substrate for the *recF* product. Two ways of producing enough of this intermediate to obtain wild-type recombination frequencies are shown: (1) derepression of ExoVIII by an *sbcA* mutation and (2) inactivation of ExoI by an *sbcB* mutation.

At present we are considering two possibilities relating the RecE and RecBC pathways. The first is that these two pathways are similar or even perhaps identical. At first this sounds like the same hypothesis rejected above. It isn't exactly since it is now based on the possibility that two or more enzymes are necessary to replace the multifunctional ExoV. Thus ExoVIII might possess an exonucleolytic activity on native DNA identical with the activity of ExoV on native DNA

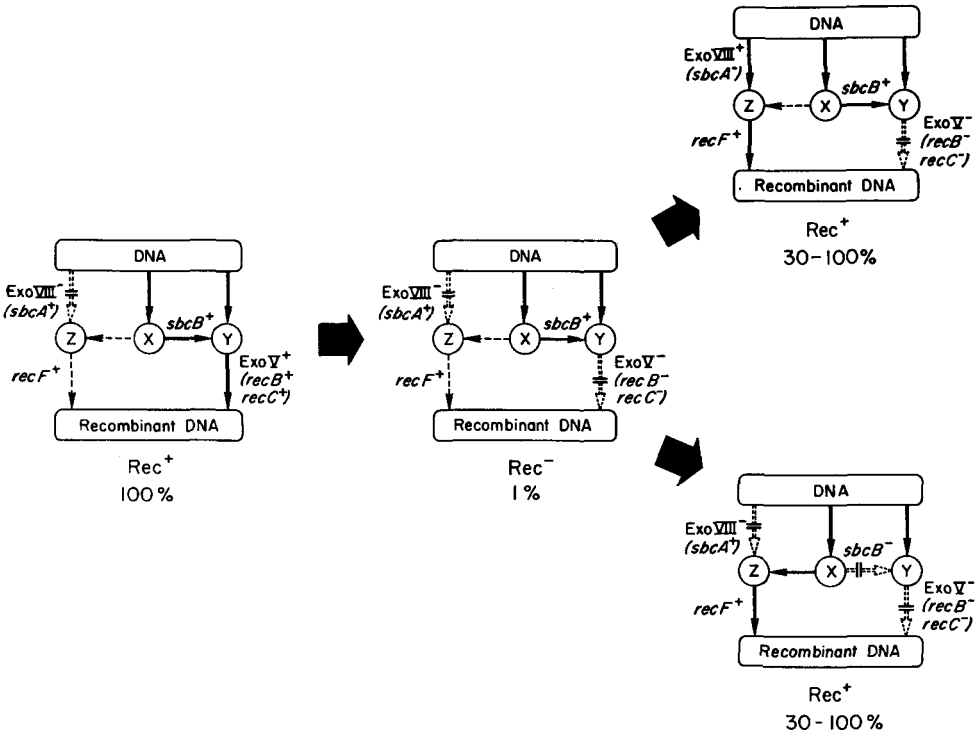

FIGURE 2.—Diagrammatic representation of hypothetical recombination pathways based on the idea that ExoV and ExoVIII are not isozymes. See the legend of Figure 1 for a description of the symbols. The very thick arrows between frames indicates the succession of changes from wild type to recombination deficient strain which has two alternatives of indirect suppression open to it for reversion to wild-type recombination proficiency.

and the *recF* product might possess an exonucleolytic or an endonucleolytic activity on denatured DNA similar to these activities of ExoV. As a result both ExoVIII and the *recF* product would participate in the RecE pathway. The second possibility is that the RecE pathway is different from the RecBC pathway. This hypothesis would be consistent with a finding that ExoVIII possesses an exonucleolytic activity on native DNA different from that of ExoV, perhaps identical to that of Exoλ.

Support for the possibility that ExoVIII acts similarly to Exoλ has been obtained by examining the effects of *recA* mutations on recombination of phage lambda in hosts which lack ExoV (i.e., are *recB⁻ recC⁻*) and contain ExoVIII (i.e., are *sbcA⁻ recE⁺*). Before looking at this evidence it is necessary to look at the effects of *recA* mutations on bacterial recombination.

It is noteworthy that a role for the *recA* gene is not indicated in Figure 2. At present it is not clear whether *recA* functions directly or indirectly in bacterial recombination. E. WITKIN during this Congress will propose that the *recA* product is necessary for induction or derepression of a set of enzymes which are directly involved in repair and mutagenesis (WITKIN 1974). It is not much of an

extension to hypothesize that *recA* regulates the synthesis of recombination enzymes. According to this hypothesis *recA* does not belong in Figure 2 as the gene determining any step of recombination; it is the genes regulated by *recA* which eventually will find their place in such a diagram. On the other hand it is possible that *recA* will be found directly involved in recombination no matter how many genes it may also regulate. One possible direct role for *recA* consistent with this view is that of determining synapsis of homologous DNA elements.

I find either the direct or indirect role of *recA* to be compatible with our findings that *recA* mutations block the RecBC, the RecF and the RecE pathways of bacterial recombination (HORII and CLARK 1973; GILLEN, SCARSELLA and CLARK, unpublished data). Ms. GILLEN's data on this point is indicated in Table 2 as a list of the frequencies of conjugational progeny inheriting donor markers from a standard Hfr strain crossed with *recA* mutants of various genotypes. In the case of the single *recA* mutant MARK GUYER has carried out an analysis of this type of cross in great detail (GUYER and CLARK, unpublished results) and finds that the maximum possible recombinant frequency to be $10^{-5}\%$ of the wild-type value. Most of the few progeny formed are F-prime strains which have inherited donor genes by repliconation (for definition of "repliconation" check CLARK 1967) and it is impossible to determine whether the F^- strains present are recombinants or revertants. When *recA*$^-$ *recB*$^-$ *recC*$^-$ *sbcA*$^-$ and *recA*$^-$ *recB*$^-$ *recC*$^-$ *sbcB*$^-$ strains are crossed with appropriate Hfr strains a few progeny are formed at frequencies as low as that observed with the *recA* single mutant. In these cases, we have not examined the progeny to determine if they are F-prime or F^- but it seems reasonable to suspect that the RecE and RecF pathways are being blocked in the absence of *recA* product as severely as is the RecBC pathway.

It is clear then that the *recA* product is necessary for the production of bacterial recombinants by the RecE pathway and we may ask whether or not it is necessary for the production of recombinants of phage lambda by the same pathway. To perform this experiment we must take account of two facts: (1) lambda determines the Exoλ which is used for its recombination and (2) lambda determines the gamma protein which inhibits the action of ExoV (UNGER and CLARK 1972; SAKAKI *et al.* 1973). Essentially therefore when wild-type *E. coli* cells are infected with wild-type lambda (i.e., *red*$^+$ *gam*$^+$) the cells become phenotypically ExoV$^-$ ExoVIII$^-$ Exoλ$^+$. Under these conditions *recA* product is not required for recombination between the A and J genes of lambda (Table 2). Nor is *recF* product required under these conditions (Table 2). The question we desire to answer is whether or not *recA* product and *recF* product are required when infected cells are ExoV$^-$ ExoVIII$^+$ Exoλ$^-$. In other words we want to know if ExoVIII will substitute for Exoλ in a *recA* and *recF* independent pathway of recombination. There are several ways of doing this experiment and at present Ms. GILLEN has performed one of them. She has infected *recB*$^-$ *recC*$^-$ *sbcA*$^-$ strains with lambda strains carrying the *bio10* substitution for a segment of lambda DNA carrying the *redX* and *gam* genes among others. Under these conditions the infected cells have the desired nuclease phenotype ExoV$^-$ ExoVIII$^+$ Exoλ$^-$ and here, too, recombination between the A and J genes of lambda is completely

independent of *recA* and *recF* (Table 2). Thus it appears that ExoVIII may be able to perform the same function in lambda recombination performed by Exoλ; in other words the enzymes may be functional isozymes.

If, when the enzymatic evidence has been obtained, Exoλ and ExoVIII turn out to be functional isozymes the question of chief interest will be what role *recA* and *recF* play in the RecE pathway of producing bacterial recombinants that they do not need to play in producing phage lambda recombinants. The possibilities that lambda may determine functional analogs of the *recA* and *recF* products or that incomplete recombinant lambda genomes may be packaged into virions and completed in the indicator bacteria seem only remotely probable since the recombination of *bio10* derivatives of lambda is *recA* dependent in $recB^+$ $recC^+$ and $recB^-$ $recC^-$ strains which are $sbcA^+$ $sbcB^+$ and it is *recF* dependent in $recB^-$ $recC^-$ $sbcB^-$ $sbcA^+$ strains. In the former strains the RecBC pathway is the major bacterial pathway of recombination; in the latter strains the RecF pathway is operating essentially exclusively.

Clearly most of the work from my own laboratory has been concerned with discovering the enzymes associated with bacterial recombination through the use of mutants. Effects of various combinations of the mutations discovered by my coworkers or by others show a high degree of complexity of interaction to produce bacterial or phage recombinants. My inclination is to use this complexity to gain insight into the possible enzymatic mechanisms involved in recombination. In addition it is possible to view this complexity at the level of the interrelationships among the realms of DNA metabolism. Such an interrelationship is particularly well brought out by the inhibitory effects of ExoV on rolling circle (i.e., sigma) replication by lambda DNA and the requirement for sigma replication or recombination for the production of mature virions of lambda replication (Enquist and Stalka 1973; Stahl *et al.* 1972a, b; Hobom and Hobom 1972).

There have been other studies of the relationships between recombination and each of the other DNA metabolic realms in both lambda-infected and in uninfected cells. A few examples will have to suffice in this limited space. Siddiqi and Fox (1973) have shown that "the insertion of [conjugationally transferred] donor material is . . . restricted to a single newly formed strand of the recipient DNA and double-strand integrations do not occur." The involvement of the *dnaB* product in conjugational recombination (Moody and Lukin 1970; Stallions and Curtiss 1971; Bednarska *et al.* 1972) implicates some type of DNA synthesis, possibly even vegetative chromosome replication, in recombination. Transductional recombination in Salmonella occurs by double-strand integration (Ebel-Tsipis, Fox and Botstein 1972). Recombination following the replication of DNA containing UV photoproducts repairs the secondary lesions (i.e., gaps) produced in the newly synthesized DNA (see e.g., Howard-Flanders 1974). UV irradiation stimulates recombination, possibly through recombinational repair and possibly also through the induction of recombination enzymes (Witkin 1973). Restriction stimulates the recombination of bacteriophage lambda (S. Lederberg personal communication). Transcription of an operon either stimu-

lates (HELLING 1967; DREXLER 1972) or inhibits (HERMAN 1968) or has no effect (SHESTAKOV and BARBOUR 1967) on recombination within that operon.

I have always found perplexing the complexities revealed by these studies and many many others on eukaryotes as well as on bacteria and viruses other than *E. coli* and lambda. Taken together with the idea that recombination occurs by a single "mechanism" in all biological situations the validity of any one of these relationships has appeared to invalidate the others. The solution to my perplexity I have found satisfying is the idea that there are multiple pathways of recombination. One view of this solution is presented in Figure 3 as a visual framework. This framework is based on a metabolic definition of recombination offered previously (CLARK 1971): "recombination is any of a set of pathways in which elements of nucleic acid interact with a resultant change of linkage of genes or parts of genes." For want of a better term "synapsis" may be used to denote the interaction involved in recombination pathways. For want of a better biochemical concept of synapsis the commonly accepted notion that synapsis involves the

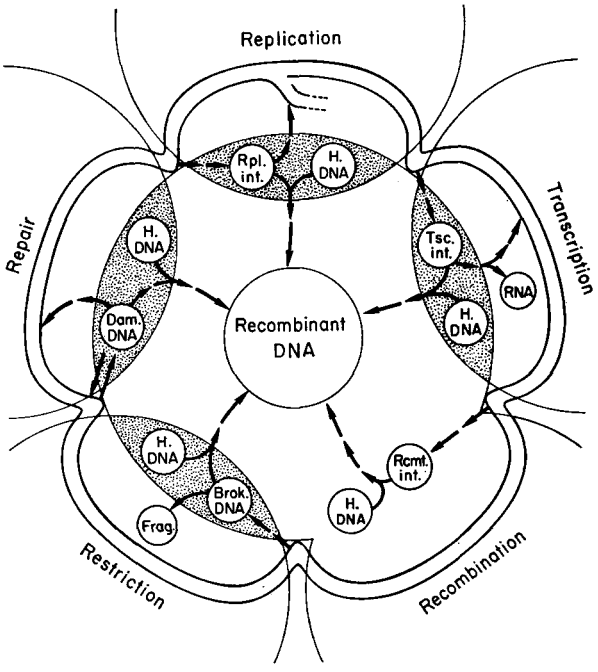

FIGURE 3.—Diagrammatic representation of hypothetical relationship among five realms of DNA metabolism. Each realm is represented as the area within a circle except for recombination which is bounded by segments of two circles. Stipled areas represent the overlaps of two realms within which reactions are common. Arrows indicate one or more steps in a pathway converting duplex chromosomal DNA to the intermediates or products shown. Reactions within each realm are represented to be acting concurrenly on a single circular chromosome with esthetically placed infoldings. "H. DNA" stands for homologous DNA; "Rpl int." for a replication intermediate; "Dam. DNA" for damaged DNA; "Brok. DNA" for broken DNA, i.e., DNA with a double-strand break; "Frag." for oligonucleotide or mononucleotide fragments of DNA; "Rcmt. int", for a recombination intermediate and "Tsc. int" for a transcription intermediate.

formation of duplex structures from locally single-stranded portions of nucleid acid may be chosen. This separates recombination pathways into presynaptic and postsynaptic portions preceding and succeeding synapsis respectively. To arrive at the framework shown in Figure 3 I have postulated that recombination as a realm of DNA metabolism will overlap the other realms of DNA metabolism to the extent that some presynaptic steps in recombination will be identical to steps in replication, repair, restriction and transcription. In these common steps single-stranded portions of DNA will presumbaly be revealed. When homologous DNA carrying complementary single-stranded sequences is available, synapsis will occur diverting the common intermediate into a recombination pathway. When no homologous DNA is available the common intermediates will continue along pathways of replication, repair, restriction or transcription. Finally in order not to exclude the possibility that some presynaptic recombination intermediates may be found to be different from those in other realms or that there may be special presynaptic enzymes of recombination or times at which recombination and none of the other types of DNA metabolism are occurring I have included this possibility in the framework.

This framework represents my best graphical effort to date and should not be construed to be strictly accurate. For example I recognize that recombinant DNA is not metabolically inert as is implied by the diagram. I also recognize that the recombinational repair pathway hypothesized by RUPP and HOWARD-FLANDERS (1968) involves replication and this is not implied by the diagram. Despite these and other flaws this particular framework permits me to envision a situation of multiple pathways of recombination occurring throughout the biological world with more or fewer steps in common with other realms of DNA metabolism depending on the enzymes present and their properties. In other words in the absence of conclusive information it permits me to envision the possibility of a metabolic interpretation of recombination.

Note added in proof: Subsequent work has indicated that the molecular weight of ExoVIII determined by glycerol gradient sedimentation is 1.3×10^5 d. (GILLEN, NAGAISHI and CLARK, unpublished data). Thus the enzyme appears to have one subunit.

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