EXTRAORDINARY RECOMBINATIONAL EVENTS IN *ESCHERICHIA COLI*. THEIR INDEPENDENCE OF THE *rec*$^+$ FUNCTION[1]

NAOMI C. FRANKLIN

Department of Biological Sciences, Stanford University, Stanford, California 94305

Received November 29, 1966

NORMAL recombinational events in bacteria are considered here to be those which occur with regularity and relatively high frequency between homologous DNA segments. Examples are the exchange of allelic genes in matings between bacteria or in generalized transduction by phage P1. In *Escherichia coli* such recombination has been found to be dependent upon the functioning of particular genes symbolized[2] *rec* (CLARK and MARGULIES 1965; HOWARD-FLANDERS and THERIOT 1966; HERTMAN and LURIA 1967).

In addition to such normal recombination, there exist in *E. coli* other genetic events more rare and perhaps more haphazard, which also result in the rejoining of DNA molecules. Two examples are the deletion of segments of the bacterial chromosome and the production of specialized transducing phage particles. We show here that these unusual recombination events occur independently of the *rec* function, suggesting that they represent events other than those that are characteristic of normal recombination. We therefore consider the possibility that they represent a different kind of recombination, occurring between substantially nonhomologous DNA segments.

MATERIALS AND METHODS

Phages: Virulent: T1 and T5.

Temperate: $i^{\lambda}c\text{I}857h^{+}\phi^{80}$, a hybrid from a cross of $\phi 80 \times$ heat-inducible $\lambda c\text{I}857$ (SUSSMAN and JACOB 1962).

$i^{\lambda}h^{-}\phi^{80}$, a hybrid from cross of $\phi 80h^{-} \times \lambda$-standard, where $\phi 80h^{-}$ is a mutant able to infect *E. coli tonB*.

$i^{\phi 80}h^{+\lambda}$, a hybrid from a cross of $\phi 80 \times \lambda$-standard.

Both hybrid phage types localize on the *E. coli* chromosome near *tonB* (controlling sensitivity to phage T1) and the *tryp* operon (controlling the synthesis of tryptophan) (Figure 1), as is most clearly demonstrated by their ability to transduce *tryp* but not *gal* (controlling galactose utilization) (FRANKLIN, DOVE and YANOFSKY 1965).

The term "lambdoid" is used in reference to temperate phages with the ability to recombine with λ.

Bacteria: Escherichia coli K-12 AB1157 and a nitrosoguanidine-induced Rec$^-$ derivative, AB2463 *rec-13* (HOWARD-FLANDERS and THERIOT 1966) were kindly provided by DONALD HELINSKI. The *Rec*$^-$ mutant shows high sensitivity to X-ray and ultraviolet-irradiation, normal ability to repair UV-irradiated T1, and 1000 to 5000-fold reduced ability to yield recombinants when acting as recipient in a cross with Hfr bacteria; the *rec-13* locus is linked to *his* (HOWARD-FLANDERS and THERIOT 1966). These characteristics are all common to the *recA1* mutant

[1] This research was supported by a grant from the National Science Foundation.

[2] The genetic symbols used are generally those recommended by DEMEREC *et al.* (1966).

described by CLARK and MARGULIES (1965). The CLARK and MARGULIES strains could not be used here because they are resistant to $\phi 80$, as well as to T1 and T5; but conclusions drawn from studies with those strains with respect to the effects of a *recA* mutation on prophages were considered applicable to the mutant of HOWARD-FLANDERS and THERIOT, and proved to be so in the situations encountered here.

A stable double mutant strain of *E. coli* carrying alleles *tryp A58* and *tryp B101* was used to detect low frequency *tryp* transduction. Other Tryp$^-$ bacteria were provided by the courtesy of CHARLES YANOFSKY.

Procedures: Rec$^+$ or Rec$^-$ bacteria lysogenic for i^{λ}cI857$h^+\phi^{80}$ or $i\phi^{80}h^{+\lambda}$ were selected by streaking out bacteria from the centers of plaques formed at 34°C. Since Rec$^-$ lysogens release very few phages, either spontaneously or after UV induction (BROOKS and CLARK 1967) their lysogenic state is tested by checking immunity to a clear mutant of the carried phage. Rec$^-$ lysogens for i^{λ}cI857$h^+\phi^{80}$ can be induced to produce phage by heating at 43°C for 15 minutes, just as are Rec$^-$ lysogens for λcI857 (BROOKS and CLARK 1967).

Heteroimmune induction of Rec$^-$ lysogens for $i\phi^{80}h^{+\lambda}$ was effected by superinfecting at a multiplicity of 20–50 with $i^{\lambda}h^-\phi^{80}$, diluting 40 × in broth, aerating at 25°C for 5 hours, and finally lysing with chloroform. Progeny phages with the host range of λ could then be detected on bacteria resistant to T5, which serve as host for phages with λ host range ($h^{+\lambda}$) but not for $h^+\phi^{80}$ nor $h^-\phi^{80}$ phenotypes.

RESULTS

Low frequency transduction by lysates of Rec$^+$ vs. *Rec$^-$ lysogens:* Because wild-type lambdoid prophages carried by the Rec$^-$ strain AB2463 are not inducible by UV-irradiation, a heat-inducible lambdoid phage was employed. Study of *gal* transduction by λ was made difficult by the fact that the parental Rec$^+$ strain AB1157 contains a nonreverting *gal* mutation. Therefore, a hybrid phage from a cross of heat-inducible λ × φ80 was constructed, since such hybrids are known

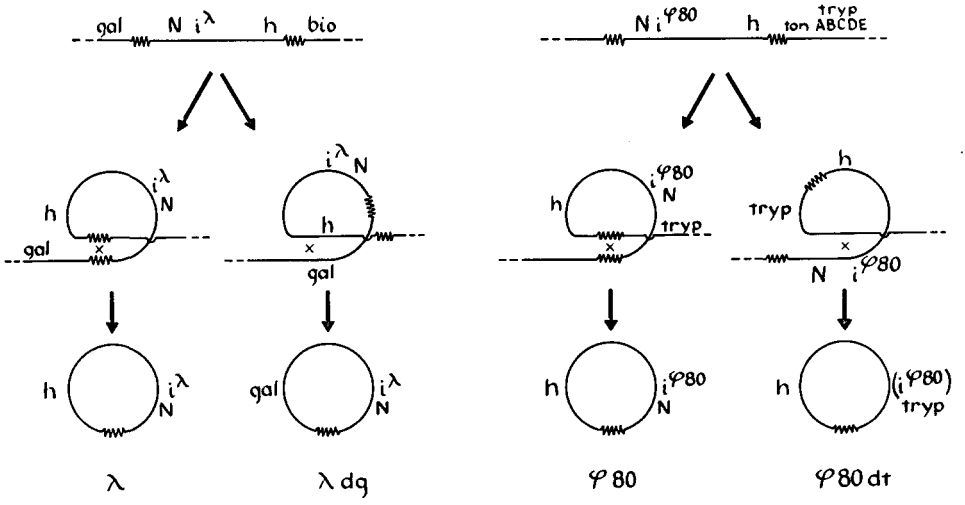

FIGURE 1.—Diagram of lysogen genomes, the excision of normal and transducing phage genomes. Based upon CAMPBELL (1962). A zigzag line represents the regions of homology between phage and bacterium. Genetic markers as given in the text. Gene orders are based upon CAMPBELL (1961), FRANKLIN, DOVE and YANOFSKY (1965), and ROTHMAN (1965).

TABLE 1

Transduction of tryp *by lysates from Rec+ or Rec⁻ lysogens*

Donor	Plaque-forming units per ml	Tryp+ transductants per ml	Tryp+ clones yielding HFT lysates
Rec+ ($i^{\lambda}c$I857$h^{+}\phi^{80}$)	1.8×10^{10}	1.7×10^{2}	2/168 = 1.2%
Rec⁻ ($i^{\lambda}c$I857$h^{+}\phi^{80}$)	3.4×10^{9}	2.3×10^{2}	0/459 <0.2%
		7.9×10^{2}	7/1592 = 0.4%
		2.9×10^{2}	3/289 = 1.0%

Transductions of a *tryp A tryp B* recipient were carried out at 34°C with heat-induced lysates prepared from the indicated donor. Tryp+ transductants were streaked on tryptophan-free media at 34°C, and liquid cultures were induced to lysis by heating to 43°C for 15 minutes. Lysates were tested for high-frequency transduction (HFT) of *tryp* by spotting onto Tryp⁻ mutants representative of the five cistrons of the *tryp* operon (ITO and CRAWFORD 1965).

to localize on the *E. coli* chromosome near *tryp* (SIGNER 1964; FRANKLIN, DOVE and YANOFSKY 1965), and to transduce *tryp* genes in a manner wholely analogous to the transduction of *gal* by λ. As can be seen in Table 1, heat-induced lysates containing $i^{\lambda}c$I857$h^{+}\phi^{80}$, whether obtained from the Rec+ or Rec⁻ strain, have the same capability for low frequency transduction of *tryp* markers.

Furthermore, equivalent numbers of high-frequency transducing (HFT) phages can be isolated from each lysate. It was found that Tryp+ clones able to produce HFT lysates could be identified on the transduction plates by their haloed appearance. The classes of HFT lysates derived from the Rec⁻ lysogen were: 3 of $A^{+}B^{+}$, 4 of $A^{+}B^{+}C^{+}$, 2 of $A^{+}B^{+}C^{+}D^{+}$ and 1 of $A^{+}B^{+}C^{+}D^{+}E^{+}$, a usual distribution (MATSUSHIRO 1963), indicating that the nature of *tryp* transduction by $i^{\lambda}h^{+}\phi^{80}$ was not altered by the *recA* mutation in the source bacteria.

Chromosomal deletions in Rec+ and Rec⁻ bacteria: Broth-grown cultures of Rec+ and Rec⁻ strains were assayed for total colony formers. About 10^{7} cells were spread on tryptone agar plates with 10^{9} T1 phages to select T1-resistant mutants (Ton⁻). Small Ton⁻ colonies result from mutations of the *tryp*-linked *tonB* gene, rather than from mutations of the *tonA* gene, as shown by their continued sensitivity to phage T5 (YANOFSKY and LENNOX 1959). The small Ton⁻ mutants were tested for simultaneous mutation to Tryp⁻ and/or to defective prophage (FRANKLIN, DOVE and YANOFSKY 1965; GRATIA 1966). defectiveness here being determined by the presence or absence of the $h^{+\lambda}$ phenotype in lysates. Table 2 shows that deletion events occur both in Rec+ and Rec⁻ bacteria. Among about 10^{8} Rec+ bacteria in two independent cultures, 3 Ton⁻ deletion mutants were detected, and among about 5×10^{7} Rec⁻ bacteria in two independent cultures, 3 Ton⁻ deletion mutants were detected, suggesting that the frequency of deletions in Rec⁻ bacteria is not very different from that in Rec+.

Low frequency recombination in the Rec⁻ strains confirmed: Recombination frequencies were measured in order to assure that the stocks used in these experiments had not lost their Rec⁻ character. In a cross with donor strain Hfr Hayes, AB2463 carrying $i^{\phi 80}h^{+\lambda}$ gave recombinants at a frequency of 10^{-4} or less, relative to AB1157 carrying $i^{\phi 80}h^{+\lambda}$.

To assure that the Ton⁻ mutations had not occurred in Rec+ revertant clones, the recombining abilities of Ton⁻-deletion mutants derived from Rec+ and Rec⁻

TABLE 2

Ton⁻ deletions from Rec+ and Rec⁻ bacteria

	Total colony-formers per ml	Ton⁻ colonies from 0.1 ml of culture			
		Total	Small, Tryp⁺	Small, Tryp⁻	Small, Tryp⁻ and defective prophage
Rec+	.....	..	6	1	..
Rec⁻	.....	..	8	1	..
Rec+ ($i^{\phi 80}h^{+\lambda}$)	2.8×10^8	90	4	1	0
		82	3	1	0
Rec⁻ ($i^{\phi 80}h^{+\lambda}$)	1.2×10^8	27	4	1	1
		34	1	1	0

were tested. The frequency of recombinants was 2×10^{-4} or less in Ton⁻Tryp⁻ strains derived from AB2463 *rec-13* as compared to Ton⁻Tryp⁻ strains derived from AB1157.

DISCUSSION

It is clear that the *rec-13* alteration has no significant effect upon the frequency of appearance either of deletions or of *tryp*-carrying transducing particles, despite the fact that the Rec⁻ characteristic reduces the frequency of normal recombination by a factor of at least 10^3 (CLARK and MARGULIES 1965; HOWARD-FLANDERS and THERIOT 1966). The meaningfulness of the absence of an effect on the appearance of transducing phages must be qualified, however, by the possibility that an enzyme of the induced phage itself could be instrumental in excising the transducing genome.

If normal recombination is not the source of deletions and specialized transducing particles, then abnormal recombination events can be postulated, consistent with the low frequency and nonspecific nature of these events, yet allowing for retention of an unbroken chromosome. Consideration of what is known of the origins of deletions and transducing particles will show that recombination between unequal (nonhomologous) DNA segments (CAMPBELL 1964) is a sufficient hypothesis to account for these events.

As a general model we refer to current interpretations of the means by which a temperate lamdboid phage (CAMPBELL 1962) or an F episome (BRODA, BECKWITH and SCAIFE 1964) is reversibly integrated into the chromosome of *E. coli* as a very frequent event. Lambda phage is believed to contain within its genome a region of recognition for a particular region within the chromosome of *E. coli*. A single recombination event between these postulated homologous regions results in the linear insertion of the previously circularized λ chromosome into the continuity of the bacterial chromosome at one particular location, giving the lysogenic state. The highly efficient release of normal lambdoid phage particles after induction of a lysogen is believed to be the reverse process: a single recombination event between the homologous termini of the prophage (CAMPBELL 1962).

Among the normal phage progeny of an induced lambdoid lysogen are rare

phage particles which include some of those bacterial genes which lie adjacent to the site of lysogenization. Such particles are responsible for the low frequency of transduction (LFT) observed for those bacterial genes. Transducing phage particles are frequently defective, in that they lack essential phage genes, in particular those phage genes which in the prophage lie distal to the bacterial genes incorporated into the transducing phage particle. Thus in the case of λ, bacterial *gal* genes replace phage genes at the host range (*h*) end of the prophage (ARBER 1958), whereas bacterial genes for biotin (*bio*) synthesis replace phage genes (N) at the immunity (*i*) end of the prophage (FUERST 1966). In the case of the λ-related phage φ80 (MATSUSHIRO 1963), bacterial *tryp* genes also replace phage genes at the immunity (*i*) end of the prophage (MATSUSHIRO 1963; personal observation) (Figure 1).

The simplest mechanism to account for the origin of transducing phages would be an exceptional excision of a DNA segment including a part of the prophage as well as bacterial DNA contiguous with it, but irrespective of prophage homologous termini. Because the ends of the excised fragment must be rejoined to give the vegetative phage genome, the most efficient means would be a single recombination event analogous to that by which normal phage is released (CAMPBELL 1962) (Figure 1). The rarity and viability of transducing phages, however, suggests that an unusual recombination event between unequal DNA segments is the basis of their appearance (CAMPBELL 1964). The frequency of LFT among normal phages in a lysate of λ or φ80 is 10^{-6} to 10^{-9}, with those bacterial genes furthest from the prophage transduced less frequently than those close by (SIGNER 1966). Transducing particles differ widely in the size of the segment of bacterial genome included (ADLER and TEMPLETON 1963; MATSUSHIRO 1963), in the amount of phage genetic material lost (ARBER 1958; CAMPBELL 1961), and in the DNA content of the resultant phage particle (WEIGLE, MESELSON and PAIGEN 1959; ADLER and TEMPLETON 1963; KAYAJANIAN and CAMPBELL 1966; MATSUSHIRO 1963). The variability suggests a randomness in the termini of these transducing particles, but the data on precise genetic end points are not sufficient to make this point strongly.

Deletions of bacterial DNA segments can also be construed as excisions of loops of chromosome by single events which rejoin the ends adjacent to the cuts. In *E. coli* it has been possible to characterize amply one set of deletions which is relatively frequent among mutations of the *tryp*-linked *tonB* gene, selected for resistance to phage T1. It was initially observed that Ton$^-$ mutants may include many Tryp$^-$ mutants (ANDERSON 1946; YANOFSKY and LENNOX 1959). Genetic characterization of these Ton$^-$Tryp$^-$ mutants showed that they contained a series of overlapping deletions extending for various distances into the *tryp* operon from that end of the operon (*tryp A*) lying closest to the *tonB* gene. As so many (about 100) point mutants of the *tryp A* gene were at hand, it was possible to characterize in some detail the termini of deletions which include only part of this gene. Among 36 deletions studied, at least 18 different deletion end points were observed within a gene which contains 801 base pairs; no obvious major hot spots for deletion end points were found (SOMERVILLE and YANOFSKY, personal communi-

cation). Scanning of the probable base sequence of the *tryp A* gene (derived from the known amino acid sequence; YANOFSKY 1967) shows there is no lengthy (>8) possible sequence of bases common to all segments within which deletions terminate, and therefore no unique base sequence is associated with deletion end points. However, since the other deletion terminus is also variable (FRANKLIN *et al.* 1965; GRATIA 1966), we cannot exclude the possibility that the two termini have substantial sequences of bases in common. We consider this unlikely, however, because it would require that the many different deletion events should result from as many instances of base sequence repeats involving a significant number of bases and occurring within a limited length of chromosome, at most 1% of the *E. coli* chromosome. THOMAS (1966) has calculated that for the entire *E. coli* chromosome repeated sequences of more the 12 bases must not occur if genetic stability is to be insured. [There remains the possibility of preferential points for deletion and transducing particle origins, as suggested on the basis of results of OZEKI (1959) and DEMEREC (1960), and further intimated by deletion patterns in *E. coli* B (YANOFSKY, personal communication), as well as in the Ton-prophage deletions of *E. coli* K-12 (FRANKLIN, *et al.* 1965).]

The mutation frequency of the *E. coli* K-12 *tryp*-linked *tonB* gene is about 10^{-6}, and the frequency of deletions among these about 5%, or 10^{-7} to 10^{-8} in the total population (YANOFSKY and LENNOX 1959; FRANKLIN, *et al.* 1965). Thus the frequency of appearance of deletions in this chromosomal region is of the same order of magnitude as the frequency of appearance of transducing phage particles.

We suggest that LFT particle formation and chromosomal deletions may be alternate results of a single type of rare event in DNA. The mechanism behind this event could be of two sorts: an error in the copying of DNA during replication, or a break-and-reunion recombination of nonhomologous DNA segments. Of these, only the recombination event has the potentiality of reciprocity, producing both the fragment and the healed deletion chromosome simultaneously. It is remarkable that such a reciprocal event has been recorded: in a particular bacterial clone an F episome appeared simultaneously with the deletion from the chromosome of the same genes now located on the episome. In the resultant cell, the episome fragment must be retained, because it carries essential genes not otherwise represented in the genome. It may very rarely reenter the chromosome at a variety of locations, but not at its initial location, a strong indication that the initial event responsible for its release was not based upon DNA homology (SCAIFE and PEKHOV 1964). Thus, this instance of F episome formation appears analogous to the generation of transducing phage particles and deletions. The reciprocity of the episomal event favors a mechanism of recombination rather than of copy error.

The rarity and variability of deletions and transducing phage particles can be interpreted to reflect the postulated lack of homology between the DNA regions recombined by these events, formally represented as exchanges between unequal DNA segments. Results here show that the functioning of the *recA* gene is not a factor in these exchanges, which conceivably are random events effected without enzymatic catalysis. That deletions are independent of the *recA* function speaks

against models for deletions which require normal recombination events (THOMAS 1966).

On the other hand, normal recombination between equal DNA segments must involve both the pairing of homologues and the joining of DNA strands from different parents, dependent upon the functioning of the gene product of *recA*, whose nature is still unknown (CLARK, CHAMBERLIN, BOYCE and HOWARD-FLANDERS 1966). It has been shown that the *recA* function is important neither to the mating of bacteria, nor to the transfer of DNA between parents, nor to the pairing potentiality of DNA from Rec$^+$ bacteria with DNA from Rec$^-$ bacteria, the latter supported by the observation that when a Rec$^-$ Hfr is mated with a Rec$^+$ F$^-$ the frequencies and times of appearance of various recombinants is normal (CLARK and MARGULIES 1965; CLARK, personal communication). We can now add the judgment that the *recA*-determined "recombinase" does not participate in recombination events between substantially nonhomologous DNA segments. Possible actions of "recombinase" would then be to facilitate the exchange of DNA strands in paired regions, or to effectuate the pairing of one duplex with another, or both.

Prediction can be made, furthermore, that other genetic events which occur with some degree of regularity will be shown to be dependent upon a *rec*$^+$ function and hence upon recombination between homologues. Possible examples are (1) the frequent deletions with a specific end point in the cysteine gene of Salmonella (DEMEREC 1960), (2) the segregation of heterogenotes, (3) the F$^+$-Hfr conversion (RICHTER, 1961), and (4) chromosome mobilization by F′ (CUZIN and JACOB 1963; PITTARD and ADELBERG 1963; SCAIFE and GROSS 1963). Indeed it has recently been shown that chromosome transfer mediated by F$^+$, colV2, colV3 or F-*lac* is greatly reduced when the donor cell is Rec$^-$ (CLOWES and MOODY 1966).

For the development of these thoughts I am appreciative of conversations with C. YANOFSKY and A. J. CLARK.

SUMMARY

Deletions of segments of the bacterial chromosome and low-frequency specialized transducing phage particles were found to occur with usual frequency in an *E. coli* strain mutated in the *recA* gene. Since this strain shows greatly reduced capacity for normal recombination between DNA homologues, it is posulated that deletions and transducing particles arise from a different kind of recombination event, occurring between substantially nonhomologous DNA segments. If, conversely, normal recombination occurs only between homologous DNA segments, then the functioning of the *recA* gene would require previously paired homologues or would itself effect pairing.

LITERATURE CITED

ADLER, J., and B. TEMPLETON, 1963 The amount of galactose genetic material in λdg bacteriophages of varying density. J. Mol. Biol. **7**: 710–720.

ANDERSON, E. H., 1946 Growth requirements of virus-resistant mutants of *Escherichia coli* strain "B." Proc. Natl. Acad. Sci. U.S. **32**: 120–128.

ARBER, W., 1958 Transduction des caractères *gal* par le bactériophage lambda. Arch. Sci. Genève **11**: 259–338.

BRODA, P., J. R. BECKWITH, and J. SCAIFE, 1964 The characterization of a new type of F-prime factor in *Escherichia coli* K12. Genet. Res. **5**: 489–494.

BROOKS, K., and A. J. CLARK, 1967 Behavior of lambda bacteriophage in a recombination deficient strain of *Escherichia coli*. J. Virol. (In press).

CAMPBELL, A. M., 1961 Sensitive mutants of bacteriophage λ. Virology **14**: 22–32. —— 1962 Episomes. Advan. Genet. **11**: 101–145. —— 1964 Transduction. pp. 49–86. *The Bacteria*, Vol. 5. Edited by I. C. GUNSALUS and R. Y. STANIER. Academic Press, New York.

CLARK, A. J., M. CHAMBERLIN, R. P. BOYCE, and P. HOWARD-FLANDERS, 1966 Abnormal metabolic response to ultraviolet light of a recombination deficient mutant of *Escherichia coli* K12. J. Mol. Biol. **19**: 442–454.

CLARK, A. J., and A. D. MARGULIES, 1965 Isolation and characterization of recombination deficient mutants of *E. coli* K-12. Proc. Natl. Acad. Sci. U.S. **53**: 451–459.

CLOWES, R. C., and E. E. M. MOODY, 1966 Chromosomal transfer from "recombination-deficient" strains of *Escherichia coli* K-12. Genetics **53**: 717–726.

CUZIN, F., and F. JACOB, 1963 Intégration réversible de l'épisome sexuel F′ chez *Escherichia coli* K12. Compt. Rend. **258**: 1350–1352.

DEMEREC, M., 1960 Frequency of deletions among spontaneous and induced mutations in Salmonella. Proc. Natl. Acad. Sci .U.S. **46**: 1075–1079.

DEMEREC, M., E. A. ADELBERG, A. J. CLARK, and P. E. HARTMAN, 1966 A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**: 61–76.

FRANKLIN, N. C., W. F. DOVE, and C. YANOFSKY, 1965 The linear insertion of a prophage into the chromosome of *E. coli* shown by deletion mapping. Biochem. Biophys. Res. Comm. **18**: 910–923.

FUERST, C., 1966 Defective biotin-transducing mutants of bacteriophage lambda. Virology **30**: 581–583.

GRATIA, J. P., 1966 Studies on defective lysogeny due to chromosomal deletion in *Escherichia coli*. I. Single lysogens. Biken's J. **9**: 77–87.

HERTMAN, I., and S. E. LURIA, 1967 Transduction studies on the role of the *Rec*+ gene in the ultraviolet induction of prophage λ. J. Mol. Biol. **23**: 117–133.

HOWARD-FLANDERS, P., and L. THERIOT, 1966 Mutants of *Escherichia coli* K-12 defective in DNA repair and in genetic recombination. Genetics **53**: 1137–1150.

ITO, J., and I. P. CRAWFORD, 1965 Regulation of the enzymes of the tryptophan pathway in *Escherichia coli*. Genetics **52**: 1303–1316.

KAYAJANIAN, G., and A. CAMPBELL, 1966 The relationship between heritable physical and genetic properties of selected *gal*⁻ and *gal*+ transducing λdg. Virology **30**: 482–493.

MATSUSHIRO, A., 1963 Specialized transduction of tryptophan markers in *Escherichia coli* K-12 by bacteriophage φ80. Virology **19**: 475–482.

OZEKI, H., 1959 Chromosome fragments participating in transduction in *Salmonella typhimurium*. Genetics **44**: 457–470.

PITTARD, J., and E. A. ADELBERG, 1963 Gene transfer by F′ strains of *Escherichia coli* K-12. II. Interaction between F-merogenote and chromosome during transfer. J. Bacteriol. **85** 1402–1408.

RICHTER, A., 1961 Attachment of wild type F factor to a specific chromosomal region in a

variant strain of *Escherichia coli*; the phenomenon of episomic alternation. Genet. Res. **2**: 333–345.

ROTHMAN, J. L., 1965 Transduction studies on the relation between prophage and host chromosome. J. Mol. Biol. **12**: 892–912.

SCAIFE, J., and J. D. GROSS, 1963 The mechanism of chromosome mobilization by an F-prime factor in *Escherichia coli* K12. Genet. Res. **4**: 328–331.

SCAIFE, J., and A. P. PEKHOV, 1964 Deletion of chromosomal markers in association with F-prime formation in *Escherichia Coli* K12. Genet. Res. **5**: 495–498.

SIGNER, E. R., 1964 Recombination between coliphages λ and φ80. Virology **22**: 650–651. —— 1966 Interaction of prophages at the *att*80 site with the chromosome of *Escherichia coli*. J. Mol. Biol. **15**: 243–255.

SUSSMAN, R., and F. JACOB, 1962 Sur un système de repression thermosensible chez le bacteriophage λ d'*Escherichia coli*. Compt. Rend. **254**: 1517–1519.

THOMAS, C. A., JR., 1966 Recombination of DNA molecules. pp. 315–337. *Progress in Nucleic Acid Research and Molecular Biology*, Vol. 5. Edited by J. N. DAVIDSON and W. E. COHN. Academic Press, New York.

WEIGLE, J., M. MESELSON, and K. PAIGEN, 1959 Density alterations associated with transducing ability in the bacteriophage lambda. J. Mol. Biol. **1**: 379–386.

YANOFSKY, C., and E. S. LENNOX, 1959 Transduction and recombination study of linkage relationships among the genes controlling tryptophan synthesis in *Escherichia coli*. Virology **8**: 425–447.

YANOFSKY, C., 1967 Colinearity of gene structure and protein structure. Scientific Amer. (In press.)