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Wild-Type Bacteriophage T4 Is Restricted by the Lambda *rex* Genes

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The bacteriophage T4 *r*II genes and the lambda *rex* (*r* exclusion) genes interact; *r*II mutants are unable to productively infect *rex*$^+$ lambda lysogens. The relationship between *rex* and *r*II has been found to be quantitative, and plasmid clones of *rex* have excluded not only *r*II mutants but T4 wild type and most other bacteriophages as well. Mutations in the T4 *motA* gene substantially reversed exclusion of T4 by *rex*.

In 1955, Seymour Benzer reported that bacteriophage T4 *r*II mutants failed to grow on *Escherichia coli* strains lysogenic for phage lambda (1). That restriction made possible the determination of the entire molecular genetics of *r*IIA and *r*IIB, including the definition of the cistron (2) and the elucidation of the triplet nature of the genetic code (6). Lambda lysogens also have been shown to restrict the growth of certain T5 and T7 mutants (15, 27). Two nonessential lambda genes, *rexA* and *rexB*, are required to restrict growth of mutants in the nonessential T4 *r*II genes (23) and of various lambdoid phages (43–45). Our work with *r*II has led us to study *rex* (39).

Little is known about the properties of the *r*II and *rex* proteins. The *r*II and possibly the *rexA* proteins are found in the membrane (19, 29, 42), and there is some evidence that the *rex* proteins may be involved in cell lysis (4). Two *r*II phenomena independent of *rex* are the rapid lysis phenotype (12) and the toxic effect of the *r*IIB polypeptide encoded by mutants FC237 and FC238 (25). These are both cell lysis phenomena and presumably involve the membrane. Indirect evidence of the importance of the *rex* genes to lambda is the fact that the only lambda genes expressed in a lysogen are *c*I, *rexA*, and *rexB*.

We subcloned *rexA* and *rexB* and placed them under the control of the *lac* and *tet* promoters. By doing so, we demonstrated that the *rex* genes are sufficient to restrict the growth of T4 *r*II mutants and that lambda *c*I is not required. We also discovered that wild-type T4 and other bacteriophages are restricted by *rex*.

MATERIALS AND METHODS

Media. Hershey broth and enriched Hershey top and bottom agar were prepared as described by Steinberg and Edgar (41). Lambda broth and plates contained 10 g of tryptone (Difco Laboratories, Detroit, Mich.) and 8 g of sodium chloride per liter.

Bacteria. We used *E. coli* NapIV, a *lacI*$^+$ B strain used extensively for T4 work (24); K strains LG90 and D1204, which are *lacI*$^-$ and *lacI*^{q}, respectively (10, 33); Bb for growing phage stocks and for crosses; and CR63 (*Su*$^+$) for growing amber mutants.

Bacteriophage. T4 *r*II were from our collection (35). The T4 *sip* and *far* mutants were provided by Dwight Hall, and the *motA* mutants *ts*G1 and *am*G1 were provided by Tom Mattson. Our *am*G1 strain was a double with the *r*II deletion NB2226, which we removed by crossing to T4 wild type. Lambda strains were from Ira Herskowitz and Peter Kuempel. Of the other phages we used, T5 came from Jim McCorquodale, T7 came from Bill Studier, and the T-even phages T2, T6, and RB69 (32) were obtained from Bill Wood.

Phage crosses. Phage crosses were performed in strain Bb at 30°C with aeration. Bacteria were grown to a concentration of approximately 2×10^8 cells per ml. Each phage was added at a multiplicity of infection of 5, for a total multiplicity of infection of 10. After a 10-min adsorption period, a sample of the adsorption mixture was diluted in chloroform to assay unadsorbed phage. Anti-T4 serum was then added to the adsorption mixture to inactivate unadsorbed phage. After 5 to 10 min in antiserum, the infected cells were diluted 10^5-fold in prewarmed H broth, and infective centers were assayed. At 75 min postinfection, chloroform was added and progeny phage were assayed.

Complementation tests. Complementation tests were performed in NapIV(prex20) at 37°C with aeration. The complementation protocol was identical to that for crosses, except that in uniparental infections the multiplicity of infection was 10.

Plasmids. Plasmid pGW7, obtained from G. Wilson, bears a fragment of lambda DNA which has the *N* gene, p_L, *rexA*, *rexB*, *c*I857, and p_R (Fig. 1a). pGW7 restricts *r*II mutants (but not wild-type T4) at low temperatures, but cells containing it grow too poorly at high temperatures to be tested. pRB6 is an adaptation of pGL101 (20) made by inserting a *Bam*HI dodecamer linker into the *Pvu*II site (36). pACYC184 (5) was provided by Mike Kahn.

RESULTS

***rex* expressed by p_{lac}.** In bacteriophage lambda (and presumably in pGW7), *c*I and the *rex* genes are transcribed from p_{RM} (30). *rexB* can also be transcribed from p_{lit} within *rexA* (28). To separate the *rex* genes from the *c*I and *N* genes and from the strong lambda promoters p_L and p_R and to make them inducible, we cloned the *Bcl*I-*Bgl*II pGW7 fragment (on which *rexA* and *rexB* are located) into the *Bam*HI site 3′ to the *lac* promoter of pRB6 (36), making prex21 (Fig. 1b).

We tested whether various T4 translation mutants that make less than wild-type amounts of *r*IIB protein (38) would grow on bacterial strains carrying prex21 (Table 1). Because of the elevated copy number of the plasmid-borne *lac* promoter, repression is incomplete in NapIV, even when expression is not induced with isopropyl-β-D-thiogalactopyranoside (IPTG). Since D1204 overproduces *lac* repressor, expression of *rex* is minimal. This results in the failure of uninduced D1204(prex21) to restrict even the *r*II deletion 638. The level of expression of *rex* in D1204 must therefore be less than that of a lambda lysogen.

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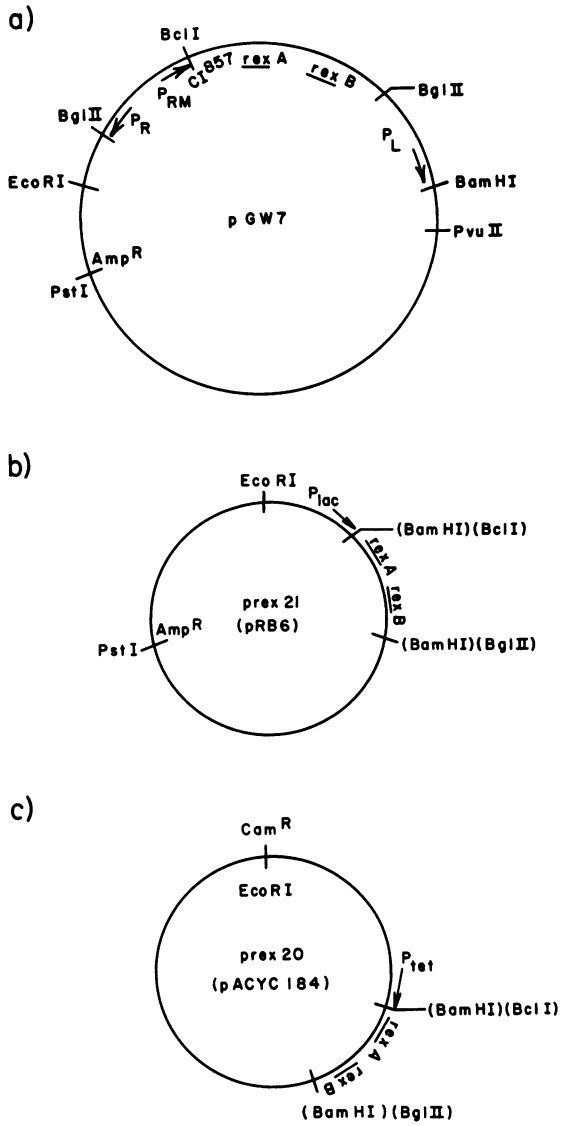

FIG. 1. (a) In pGW7, DNA from the *Eco*RI site to the *Bam*HI site was derived from bacteriophage lambda. From *Bam*HI clockwise to *Eco*RI, it was derived from pBR322. (b) prex21 was constructed by cloning the *rex* genes into pRB6 (36). (c) prex20 was constructed by cloning the *rex* genes into pACYC184. Restriction sites in parentheses were used in construction, but no longer exist.

The T4 *r*IIB translation mutants are restricted in rough correspondence to the amount of *r*IIB protein they make, as measured at early times during phage infection at the same temperatures (37, 38). The *r*IIB translation data for 30°C are given in Table 1. Restriction was greater at higher temperatures and as *rex* expression was increased by addition of IPTG. Independent of induction, restriction was also a function of bacterial strain used. The data also showed that *c*I protein was not required for *rex* activity, a formal possibility noted by Toothman and Herskowitz (43). The unprecedented result was that T4 wild type was restricted by LG90(prex21).

Increased restriction by prex20. prex20 was constructed on the chance that *rex* expression would be increased by using the *tet* promoter of pACYC184. The same *Bcl*I-*Bgl*II DNA fragment was cloned downstream from the *tet* promoter at the *Bam*HI site of pACYC184 (Fig. 1c). Cells carrying prex20 were more restrictive for T4 wild type than were the same strains carrying prex21. LG90(prex20) restricted wild-type T4 at 30, 37, and 42°C and also restricted *r*II translation and null mutants. The efficiency of plating of $T4^+$ on LG90(prex20) at 30°C was about 2×10^{-4}. On NapIV (prex20), it was 10^{-1}, and on NapIV(prex21), with or without IPTG, it was about 1. As with prex21, the B strain NapIV mediated considerably less restriction than LG90. When there was a large number of phage on a plate, restriction was visibly more stringent at higher temperatures: phage growth was tightly restricted at 42°C, a bit leaky at 37°C, and leakier at 30°C. This may reflect the effect of temperature on the mechanism of restriction or on the relative amounts of *rex* or other gene products. As expected from the results of Garen (9), the exclusion of wild-type T4 by prex20 was substantially reversed by the addition of magnesium (data not shown).

Mutants that grow on prex20-containing strains. The efficiency of plating on LG90(prex20) of phage from a $T4^+$ lysate, about 2 in 10^4, was consistent with the idea that phage that grow contain spontaneous mutations of a T4 gene. Because *motA* mutations had been shown to increase the viability of an *r*II deletion grown on K(λ) (13), we tested the *motA* mutant *ts*G1 (21) by plating it on LG90(prex20). *ts*G1 affects the expression of early genes, causing synthesis of less than wild-type amounts of some, but overproduction of others (22). At 30°C, it plated with an efficiency of 0.3 on LG90(prex20). The other *motA* mutants, *am*G1, *farP14*, *farP85*, *sip-1*, and *sip-2*, were tested to see whether growth on prex20 is allele specific. All had efficiencies of plating of 0.3 to 0.8 on LG90(prex20) at 30°C. The six *motA* mutants tested were isolated at different times by different research

TABLE 1. Restriction of *r*II quantity mutants by prex21-containing bacteria[a]

T4 translation mutants	% of wild-type *r*IIB at 30°C	Restriction by strain:								
		D1204 (*lacI*^{q}) (all temps)	NapIV (*lacI*$^+$) at:			NapIV (*lacI*$^+$) + IPTG at:			D1204 (*lacI*^{q}) + IPTG at 42°C	LG90 (*lacI*$^-$) at 42°C
			20°C	30°C	42°C	20°C	30°C	42°C		
$T4^+$	100	+	+	+	+	+	+	+	+	−
*rz*HA87	46	+	+	+	+	+	+	+	−	−
*rz*EM72	39	+	+	+	−	+	+	−	−	−
*rz*AP10	27	+	+	+	−	+	+	−	−	−
*r*HD263	7	+	+	−	−	−	−	−	−	−
*rz*P18	3	+	−	−	−	−	−	−	−	−
*r*638	0	+	−	−	−	−	−	−	−	−

[a] Bacteriophage (10^8/ml) were spotted on a lawn of fresh plasmid-bearing bacteria on EHA plates. Where indicated, 50 μl of 0.1 M IPTG was added to the top agar. +, Clearing of the spot; −, no significant effect on the lawn. All of the phages tested grew on the parent strains at all temperatures. *r*IIB quantity data are from Singer et al. (37, 38). *r*638 is a deletion of the entire *r*IIB cistron.

groups, but had been found to have somewhat similar phenotypes and genetic map positions (11).

We noted that the deletion *Sa*9 grew as poorly as an *r*II mutant on LG90(prex20) and prevented rescue of the infection by mutation in *motA*. Subsequently, we measured efficiencies of plating, or mutation indices, of mutants in the region (Table 2), particularly the *Sa*Δ series (7). We found that the gene required for rescue was *ndd*, which is the "nonessential" T4 gene responsible for disruption of the *E. coli* nucleoid and for early shutoff of host DNA synthesis (16, 40). This identification was verified by crossing the *motA* mutants *rim-2*, *rim-13*, and *am*G1 (see below) to *Sa*Δ5 and *Sa*Δ3. The double mutants *motA-Sa*Δ5 would not grow on LG90(prex20), whereas the *motA-Sa*Δ3 doubles would. *Sa*Δ5 deletes parts or all of *ndd*, *stp*, and *ac*, whereas *Sa*Δ3 covers the 3′ end of *stp* and the 5′ end of *ac* (7).

New *motA* mutants. We made stocks of two of the phages which grew when we plated a wild type lysate on LG90 (prex20) at 30°C. These spontaneous T4 mutants are called *rim-2* and *rim-13* (*rex* immunity). We noted that none of the other *motA* mutants grew on a prex host with the virulence of *rim-2*. *rim-13* was temperature sensitive and in spot tests phenotypically resembled *ts*G1.

To genetically map the *rim* mutants, we performed complementation tests and genetic crosses. If the *rim* mutants and *am*G1 were recessive and in the same gene, then they would fail to complement each other and hence would produce progeny phage on mixed infection of the restrictive host, NapIV(prex20). If they were recessive and in different genes, then they would complement each other and consequently fail to yield progeny in coinfections.

In single-cycle growth in NapIV(prex20) at 37°C, *rim-2*, *rim-13*, *am*G1, and T4$^+$ had burst sizes of 239, 147, 12.3, and 0.068, respectively (Table 3). In mixed infection with wild type, the burst sizes of *rim*-2, *rim*-13, and *am*G1 were reduced to 7.8, 4.5, and 0.62 (Table 3), respectively, indicating that each mutant is recessive. In mixed infection with each other, the burst sizes were *rim-2–rim-13*, 191; *rim-2–am*G1, 144; and *rim-13–am*G1, 87.4 (Table 3). Thus, the mutants failed to complement one another and therefore were in the same functional unit or cistron. Neither the *rim* mutants nor *am*G1 yielded progeny in mixed infection with the gene 43 amber mutant *am*B22 (Table 3).

From the conclusion that the *rim* mutants were in *motA* it can be predicted that they are linked to the *ac* locus, are distant from gene 43, and are closely linked to *am*G1 and to each other. To verify these predictions, we crossed *rim-2*, *rim-13*, and *am*G1 to *ac*41, *am*B22, and each other. The *rim* mutants and *am*G1 were linked to *ac*41 (Table 4, column 2), unlinked to *am*B22 (column 3), and tightly linked to each other (columns 4 and 5). The frequencies of wild-type recombinants in *rim* × *rim* and *rim* × *am*G1 were as low as or lower than those observed by Mattson et al. (22) for *am*G1 × *ts*G1 crosses.

Other bacteriophages. We have tested a variety of bacteriophages to see whether they are restricted by plasmids expressing *rex* (Table 5). T2 and T6 are T-even phages closely related to T4. RB69 is a T-even phage which has the unusual property of excluding T4 in mixed infections (32). T7*rbl* is a T7 mutant, mapping in gene 5.7 (8), which does not grow on *rex*$^+$ lambda lysogens (27). The lambda strains tested are a small representative sample of those tested for

TABLE 2. Efficiencies of plating or mutation indices on LG90(prex20) at 30°C[a]

T4 strain	Efficiency of plating
T4$^+$	7.6×10^{-5}
rim-2	0.71
rim-13	0.72
*am*G1	0.50
*Sa*Δ9	$<1.0 \times 10^{-8}$
*Sa*Δ3	5.0×10^{-4}
*Sa*Δ4	$<1.1 \times 10^{-7}$
*Sa*Δ5	$<2.7 \times 10^{-7}$
ndd (98 × 3)	$<8.2 \times 10^{-9}$
*r*1589	$<5 \times 10^{-8}$

[a] Efficiencies of plating or indices of mutation are the ratios of a phage titer on LG90(prex20) at 30°C on EHA plates to its titer on CR63 at 37°C on EHA plates. If a phage grows on both bacteria (i.e., *rim-2*), the ratio represents the relative efficiency of plaque-forming ability. If a phage is unable to grow on LG90(prex20) (e.g., T4$^+$), the ratio approximates the fraction of phage in the stock that has gained the ability to grow on LG90(prex) because of mutation. Plating cultures were prepared by diluting overnight cultures 100-fold on H broth and growing them at 37°C with aeration for approximately 3 h, after which time they were kept on ice. LG90(prex20) cultures were grown in the presence of 25 μg of chloramphenicol per ml.

TABLE 3. Burst size data from single-cycle growth and complementation tests with NapIV(prex20) at 37°C

Infecting phage	Burst size[a]
rim-2	239
rim-13	147
*am*G1	12.3
T4$^+$	0.068
*am*B22	<0.018
rim-2–T4$^+$	7.8
rim-13–T4$^+$	4.5
*am*G1–T4$^+$	0.62
*am*B22–T4$^+$	<0.018
rim-2–rim-13	191
*rim-2–am*G1	144
*rim-13–am*G1	87.4
*rim-2–am*B22	3.9
*rim-13–am*B22	1.4
*am*G1-*am*B22	0.28

[a] Average number of progeny phage yielded per infected bacterium. The data for uniparental infections, except for *am*B22, are the averages of four experiments, those for mixed infection with wild type or *rim-rim* or *rim-am*G1 are the averages of two experiments, and those involving *am*B22 are from a single set of experiments.

TABLE 4. Recombination data from crosses involving *rim-2*, *rim-13*, and *am*G1

Parent 1	% Recombination[a] with parent 2			
	*ac*41	*am*B22	*rim-2*	*rim-13*
rim-2	4.1	28.4		
rim-13	5.2	35.3	1.6	
*am*G1	8.8	25.0	0.4	<0.6

[a] Percent recombination is 200 times the frequency of wild-type recombinants for crosses involving *am*B22 and crosses between *rim* mutants or *rim* and *am*G1. In these crosses, progeny were plated on CR63, and individual plaques were tested for ability to grow on S/6 and LG90(prex20); wild-type recombinants grew on the former but not on the latter strain. The values for *rim-2* × *rim-13*, *rim-2* × *am*G1, and *rim-13* × *am*G1 are based on 4 of 506, 1 of 496, and 0 of 344 recombinants, respectively, per total tested. For crosses involving *ac*41, *ac*41/*rim*, or *ac*41-*am*G1, double mutants were selected on LG90(prex20) supplemented with 2 μg of acriflavin per ml. The frequencies of recombinants in these crosses were corrected for the efficiency of plating of *ac*41–*rim-2*, *ac*41–*rim-13*, and *ac*41-*am*G1 double mutants and then multiplied by 200.

TABLE 5. Restriction of various bacteriophages by *rex*[a]

Bacteriophage	Restriction by strain:								
	LG90 at:			LG90(prex21) at:			LG90(prex20) at:		
	30°C	37°C	43°C	30°C	37°C	43°C	30°C	37°C	43°C
T4$^+$	+	+	+	+	−	−	−	−	−
*ts*G1	+	+	+	+	+	−	−	−	−
*am*G1	+	+	+	+	+	−	+	−	−
rim-2	+	+	+	+	+	+	+	+	−
rim-13	+	+	+	+	+	−	−	−	−
*r*638	+	+	+	−	−	−	−	−	−
T2$^+$	+	+	−	−	−	−	−	−	−
T5$^+$	+	+	+	+	−	−	+	−	−
T6$^+$	+	+	+	+	+	−	−	−	−
T7$^+$	+	+	+	+	+	+	+	+	−
rbl	+	+	+	−	−	−	−	−	−
RB69$^+$	+	+	+	−	−	−	−	−	−
Lambda$^+$	+	+	+	+	+	+	+	+	+
*imm*434	+	+	+	+	+	+	+	+	−
*imm*434*c*I60	+	+	+	+	+	+	+	+	−
*imm*434*c*I6T	+	+	+	+	+	+	+	+	−
*imm*434(OP)80	+	+	−	+	+	−	−	−	−
*imm*434*c*I60(OP)P22	+	+	+	+	+	−	−	−	−
*imm*434*c*I6T*nin*5	+	+	+	+	+	−	−	−	−
*imm*434*c*I6T*ren*51	+	+	+	+	+	−	−	−	−

[a] Bacteriophage (10^4) in 20 μl of saline were spotted on a lawn of bacteria from a fresh overnight culture grown in lambda broth plus 0.2% maltose, with an antibiotic where appropriate. All plates were warmed while the spots were being applied and until the spots were dry. Warming the plates significantly increases the stringency of restriction at higher temperatures, but probably results in overstating restriction at the lowest incubation temperature (30°C). +, Clearing of the spot; −, no significant effect on the lawn. These spot tests were done with lambda plating medium. The nonlambdoid phages were also tested under standard T4 plating conditions (H broth cultures and EHA top and bottom agar), and the same results were obtained. Under those conditions, none of the lambdoid phages would grow well, even on the parent LG90 strain, unless magnesium was added to the medium.

rex-mediated exclusion by Toothman and Herskowitz (43–45). Only wild-type lambda was unaffected by our most restrictive conditions. Of the other phages, T7 was the most resistant to *rex* restriction, although its characteristic large plaque was diminished by the *rexA* and *rexB* proteins. The susceptibilities of the lambdoid phage to *rex* exclusion support the results of Toothman and Herskowitz (44): OP region substitutions and the *nin*-5 and *ren* mutations are the most readily excluded.

DISCUSSION

***rex* restriction of T4.** The data indicate that *rex* exclusion of T4 is a quantitative phenomenon. *rex* expression was varied by promoter and induction. *r*IIB synthesis was reduced by translation mutants. The *rex* restriction was tighter at higher temperatures, and whereas it seems likely that this was an aspect of the physiology of the *rex* proteins, an effect of temperature on expression cannot be ruled out. Most important, high-level *rex* expression restricts T4 *r*II$^+$ growth.

rex exclusion was relieved if the T4 phage was *motA*. We used this characteristic to select two new *motA* mutants. The *motA* (modifier of transcription) gene product activates T4 middle-mode transcription (21, 22). The sequence a/ta/t TGCTTc/tA in the −30 region of a T4 promoter has been identified as necessary for middle-mode transcription (3; N. Guild, M. Gayle, R. Sweeney, T. Walker, T. Modeer, and L. Gold, J. Mol. Biol., in press). It is possible that *motA* phage grow on cells harboring prex20 because middle-mode transcription is defective and the levels of some early T4 transcripts are elevated (22; N. Guild et al., in press). That is, a complex redistribution of prereplicative transcripts overcomes *rex* restriction. Growth could occur because of underproduction of *r*IIB and overproduction of *r*IIA. (In this model, the overproduction of *r*IIA, rather than just the ratio of *r*IIA to *r*IIB, is important: *r*II mutants which only reduce *r*IIB synthesis do not grow as well as wild type.) It is also possible that *motA*-controlled genes other than *r*II are involved.

The dysfunction of *motA* protein may relieve *rex* restriction directly, perhaps because the *motA* protein has a direct role in T4 DNA replication. T4 appears to utilize different types of replication origins (17, 18). The *motA* protein almost certainly binds to DNA (N. Guild et al., in press), appears very early in infection (46), and could function during replication by binding to DNA at *motA* "boxes." *motA* mutants may be able to escape restriction by *rex* gene products by switching to an alternate replication initiation pathway.

***rex* restriction of lambda and other phages.** Of the lamboid phage we tested, only wild-type lambda resisted exclusion by *rex* under all of the experimental conditions. The exclusion of lambdoid phage with OPQ-region mutations repeated the findings of Toothman and Herskowitz (44) that these phage are susceptible to *rex*. The exclusion of the various *imm*434 strains under very restrictive conditions (Table 5) is a new observation, possibly analogous to *rex* restriction of wild-type T4.

Every bacteriophage tested, other than *rex*-homologous lambda, could be excluded by *rex* expressed in cells whose own replication appeared to be proceeding quite normally. In T4 *r*II infections, the *rex*-mediated block to phage growth occurs relatively late, just after the initial replication of phage DNA (9, 34). *rex* interference with many bacteriophages implies that they may be affected at a common stage of DNA replication.

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LITERATURE CITED

1. **Benzer, S.** 1955. Fine structure of a genetic region in bacteriophage. Proc. Natl. Acad. Sci. USA **41**:344–354.
2. **Benzer, S.** 1957. The elementary units of heredity, p. 70–93. *In* W. D. McElroy and B. Glass (ed.), *The chemical basis of heredity*. The Johns Hopkins Press, Baltimore.
3. **Brody, E., D. Rabussay, and D. H. Hall.** 1983. Regulation of transcription of prereplicative genes, p. 174–183. *In* C. K. Mathews, E. M. Kutter, G. Mosig, and P. G. Berget (ed.), *Bacteriophage T4*. American Society for Microbiology, Washington, D.C.
4. **Campbell, J. H., and B. G. Rolfe.** 1975. Evidence for a dual control of the initiation of host-cell lysis caused by phage lambda. Mol. Gen. Genet. **139**:1–8.
5. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**:

1141–1156.
6. **Crick, F. H. C., L. Barnett, S. Brenner, and R. J. Watts-Tobin.** 1961. General nature of the genetic code for proteins. Nature (London) **192**:1227–1232.
7. **Depew, R. E., T. J. Snopek, and N. R. Cozzarelli.** 1975. Characterization of a new class of deletions of the D region of the bacteriophage T4 genome. Virology **64**:144–152.
8. **Dunn, J. J., and F. W. Studier.** 1983. Complete nucleotide sequence of bacteriophage T7 DNA and the locations of T7 genetic elements. J. Mol. Biol. **166**:477–535.
9. **Garen, A.** 1961. Physiological effects of rII mutations in bacteriophage T4. Virology **14**:151–163.
10. **Guarente, L., G. Lauer, T. M. Roberts, and M. Ptashne.** 1980. Improved methods for maximizing expression of a cloned gene: a bacterium that synthesizes rabbit beta-globin. Cell **20**:543–553.
11. **Hall, D. H., and R. D. Snyder.** 1981. Suppressors of mutations in the rII gene of bacteriophage T4 affect promoter utilization. Genetics **97**:1–9.
12. **Hershey, A. D.** 1946. Mutation of bacteriophage with respect to type of plaque. Genetics **31**:620–640.
13. **Homyk, T., A. Rodriguez, and J. Weil.** 1976. Characterization of T4 mutants that partially suppress the inability of T4rII to grow in lambda lysogens. Genetics **83**:477–487.
14. **Homyk, T., and J. Weil.** 1974. Deletion analysis of two nonessential regions of the T4 genome. Virology **61**:505–523.
15. **Jacquemin-Sablon, A., and Y. T. Lanni.** 1973. Lambda-repressed mutants of bacteriophage T5. I. Isolation and genetical characterization. Virology **56**:230–237.
16. **Koerner, J. F., and D. P. Snustad.** 1979. Shutoff of host macromolecular synthesis after T-even bacteriophage infection. Microbiol. Rev. **43**:199–223.
17. **Kreuzer, K. N., and B. M. Alberts.** 1985. A defective phage system reveals bacteriophage T4 replication origins that coincide with recombination hot spots. Proc. Natl. Acad. Sci. USA **82**:3345–3349.
18. **Kreuzer, K. N., and B. M. Alberts.** 1986. Characterization of a defective phage system for the analysis of bacteriophage T4 DNA replication origins. J. Mol. Biol. **188**:185–198.
19. **Landsmann, J., M. Kroger, and G. Hobom.** 1982. The rex region of bacteriophage lambda: two genes under three-way control. Gene **20**:11–24.
20. **Lauer, G., R. Pastrana, J. Sherley, and M. Ptashne.** 1981. Construction of overproducers of the bacteriophage 434 repressor and cro proteins. J. Mol. Appl. Gen. **1**:139–148.
21. **Mattson, T., J. Richardson, and D. Goodin.** 1974. Mutant of bacteriophage T4D affecting expression of many early genes. Nature (London) **250**:48–50.
22. **Mattson, T., G. van Houwe, and R. H. Epstein.** 1978. Isolation and characterization of conditional lethal mutations in the mot gene of bacteriophage T4. J. Mol. Biol. **126**:551–570.
23. **Matz, K., M. Schmandt, and G. N. Gussin.** 1982. The rex gene of bacteriophage lambda is really two genes. Genetics **102**:319–327.
24. **Nelson, M. A., M. Ericson, L. Gold, and J. F. Pulitzer.** 1982. The isolation and characterization of TabR bacteria: hosts that restrict bacteriophage T4 rII mutants. Mol. Gen. Genet. **188**:60–68.
25. **Nelson, M. A., B. S. Singer, L. Gold, and D. Pribnow.** 1981. Mutations that detoxify an aberrant T4 membrane protein. J. Mol. Biol. **149**:377–403.
26. **O'Farrell, P., and L. M. Gold.** 1973. Bacteriophage T4 gene expression: evidence for two classes of prereplicative cistrons. J. Biol. Chem. **248**:5502–5511.
27. **Pao, C. C., and J. F. Speyer.** 1975. Mutants of T7 bacteriophage inhibited by lambda prophage. Proc. Natl. Acad. Sci. USA **72**: 3642–3646.
28. **Pirrotta, V., K. Ineichen, and A. Walz.** 1980. An unusual RNA polymerase binding site in the immunity region of phage lambda. Mol. Gen. Genet. **180**:369–376.
29. **Pribnow, D., D. C. Sigurdson, L. Gold, B. S. Singer, C. Napoli, J. Brosius, T. J. Dull, and H. F. Noller.** 1981. rII cistrons of bacteriophage T4 DNA sequence around the intercistronic divide and positions of genetic landmarks. J. Mol. Biol. **149**: 337–376.
30. **Ptashne, M., K. Backman, M. Z. Humayun, A. Jeffrey, R. Maurer, B. Meyer, and R. T. Sauer.** 1976. Autoregulation and function of a repressor in bacteriophage lambda. Science **194**:156–161.
31. **Pulitzer, J. F., A. Coppo, and M. Caruso.** 1979. Host-virus interactions in the control of T4 prereplicative transcription. II. Interaction between tabC (rho) mutants and T4 mot mutants. J. Mol. Biol. **135**:979–997.
32. **Russell, R. L., and R. J. Huskey.** 1974. Partial exclusion between T-even bacteriophages; an incipient genetic isolation mechanism. Genetics **78**:989–1014.
33. **Sadler, J. R., M. Tecklenberg, and J. L. Betz.** 1980. Plasmids containing many tandem copies of a synthetic lactose operator. Gene **8**:279–300.
34. **Sauerbier, W., S. M. Puck, A. R. Bräutigam, and M. Hirsch-Kauffmann.** 1969. Control of gene function in bacteriophage T4. I. Ribonucleic acid and deoxyribonucleic acid metabolism in T4rII-infected lambda-lysogenic hosts. J. Virol. **4**:742–752.
35. **Shinedling, S., B. S. Singer, M. Gayle, D. Pribnow, E. Jarvis, B. Edgar, and L. Gold.** 1987. Sequences and studies of bacteriophage T4 rII mutants. J. Mol. Biol. **195**:471–480.
36. **Shinedling, S., L. T. Walker, and L. Gold.** 1986. Cloning the complete *r*IIB gene of bacteriophage T4 and some observations concerning its middle promoters. J. Virol. **60**:787–792.
37. **Singer, B. S., and L. Gold.** 1976. A mutation that confers temperature sensitivity on the translation of rIIB in bacteriophage T4. J. Mol. Biol. **103**:627–646.
38. **Singer, B. S., L. Gold, S. T. Shinedling, M. Colkitt, L. R. Hunter, D. Pribnow, and M. A. Nelson.** 1981. Analysis in vivo of translational mutants of the rIIB cistron of bacteriophage T4. J. Mol. Biol. **149**:405–432.
39. **Singer, B. S., S. T. Shinedling, and L. Gold.** 1983. The rII genes: a history and a prospectus, p. 327–333. *In* C. K. Mathews, E. M. Kutter, G. Mosig, and P. B. Berget (ed.), *Bacteriophage T4*. American Society for Microbiology, Washington, D.C.
40. **Snustad, P. D., C. J. H. Bursch, K. A. Parson, and S. H. Hefeneider.** 1976. Mutants of bacteriophage T4 deficient in the ability to induce nuclear disruption: shutoff of host DNA and protein synthesis, gene dosage experiments, identification of a restrictive host, and possible biological significance. J. Virol. **18**:268–288.
41. **Steinberg, C. M., and R. S. Edgar.** 1962. A critical test of a current theory of genetic recombination in bacteriophage. Genetics **47**:187–208.
42. **Takacs, B. J., and J. P. Rosenbusch.** 1975. Modification of *Escherichia coli* membranes in the prereplicative phase of phage T4 infection. J. Biol. Chem. **250**:2339–2350.
43. **Toothman, P., and I. Herskowitz.** 1980. Rex-dependent exclusion of lambdoid phages. I. Prophage requirements for exclusion. Virology **102**:133–146.
44. **Toothman, P., and I. Herskowitz.** 1980. Rex-dependent exclusion of lambdoid phages. II. Determinants of sensitivity to exclusion. Virology **102**:147–160.
45. **Toothman, P., and I. Herskowitz.** 1980. Rex-dependent exclusion of lambdoid phages. III. Physiology of the abortive infection. Virology **102**:161–171.
46. **Uzan, M., J. Leautey, Y. de'Aubenton-Carafa, and E. Brody.** 1983. Identification and biosynthesis of the bacteriophage T4 mot regulatory protein. EMBO J. **2**:1207–1212.