Requirement for Protein Synthesis for Survival of Unmodified Bacteriophage T1 in a Restricting Host

JEROO S. KOTVAL†* AND J. R. CHRISTENSEN

Department of Microbiology, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

At high multiplicities of infection, a substantial fraction of restricting cells (P1 lysogens) could be productively infected by unmodified coliphage T1 (T1.0) provided that protein synthesis was uninhibited during the first 5 min of infection. Successful infection under restricting conditions was accompanied by more genetic recombination than was seen under nonrestricting conditions. When protein synthesis was inhibited during early infection of a restricting host, the recombination frequency declined for markers on T1.0 genomes; no effect was seen on recombination between markers on modified (T1.P) genomes. This suggested that recombination between unmodified genomes may be essential for their survival under conditions of host restriction. In a restricting host, genetic markers on T1.0 could recombine with T1.P even when the rescuing phage was added 6 min after T1.0 infection. However, even marker rescue recombination was diminished when protein synthesis was inhibited during early infection. Since DNA restriction is an early event, protein synthesis may be required soon after infection of a restricting host by T1.0 in order to preserve restriction-damaged DNA in a form that can participate in recombination. Experiments are also described that rule out some possibilities for the role of such a protein(s).

The restriction and modification system of the prophage P1 operates on bacteriophage T1 DNA (8). For several restriction and modification systems, it has been shown that within minutes after entry a part of the unmodified DNA is degraded to acid-soluble (low-molecular-weight) products (1). Despite nucleolytic degradation of T1 DNA after P1-directed restriction, a significant fraction of the incoming phage DNA survives degradation (10). This has been shown to have several genetic effects. For most of the T1 genes tested, unmodified T1 (T1.0) can complement the growth of coinfecting or superinfecting amber mutants of modified T1 (T1.P) when the host is the nonpermissive and restricting strain *Escherichia coli* B(P1) (3). After infection of P1 lysogens by T1.0, genetic markers can be rescued by coinfection or by superinfection by T1.P even when this superinfection is delayed for up to 1 h (2, 4). When T1.0 is present at a high multiplicity of infection (MOI), a substantial fraction of the population of restricting cells can be productively infected (6, 7, 10). This process, referred to as cooperative infection, is accompanied by an increase in recombination frequency between genetic markers (6, 7). The recombination occurring under these conditions has been referred

to as cooperative recombination (7). It is independent of the host recombination genes under *recA* control (7). Certain physiological conditions must be assured during the first few minutes after T1 infection in order for modified phage at high MOI to productively infect a substantial fraction of restricting cells. These include aeration, the presence of nutrient medium, and uninhibited protein synthesis (6, 10).

A superficial examination of these data might lead to the conclusion that the survival of DNA, the genetic continuity of which is interrupted by restriction, merely requires immediate recombination to form complete phage genomes that would be protected from nuclease activity *in vivo*. The experiments reported in this paper were undertaken to investigate whether the immediate need for protein synthesis is solely a reflection of the need for recombination-related functions in order for restriction-damaged T1 DNA to survive. The results suggest that after infection by T1.0, a protein or set of proteins is rapidly induced which facilitates the preservation of restriction-damaged DNA in a form that can participate in recombination even after a delay.

MATERIALS AND METHODS

Phage strains, bacterial strains, and media. Bacterial strains *E. coli* B and *E. coli* B(P1) have been previously described (4). All of the phage crosses re-

† Present address: Department of Microbiology and Immunology, The Albany Medical College of Union University, Albany, NY 12208.

ported here were between T1 Sb Hr Kn⁺ and T1 Sb⁺ Hr⁺ Kn. These phage strains carry plaque morphology markers and have been described previously (6, 7). The media used for preparing log-phase cells for performing phage crosses and for scoring the progeny phage also have been described previously (4, 7).

Procedures for phage crosses and for scoring markers. The preparation of log-phase cells for T1 attachment has been previously described (10). Procedures described by Kotval et al. (7) were used for performing phage crosses and for scoring the plaque morphology markers.

Protein synthesis inhibition. Chloramphenicol (CAM) (Sigma Chemical Co.) was added to give a final concentration of 50 µg/ml. CAM was added to log-phase cells at 37°C 8 min before addition of the phage mixture unless otherwise stated. It was removed by centrifugation of the cell suspension and further suspension in drug-free medium 5 min after phage infection unless otherwise stated. In all other respects, the cells were treated according to the procedures described previously (7).

RESULTS

Effect of inhibition of protein synthesis on cooperative infection and on recombination occurring in the presence and absence of host restriction. Cooperative infection of P1 lysogens by T1.0 is characterized by a sharply increased proportion of bacteria successfully infected and by an accompanying increase in the proportion of progeny phage that are genetically recombinant (6, 7). Potts and Christensen (10) showed that the presence of CAM during the first few minutes after infection is sufficient to prevent the enhanced level of successful infection of restricting cells under cooperative conditions. In those experiments, the

success of the cooperative infection was judged from the proportion of infected cells that produce T1.P (modification ratio). We examined this phenomenon further, with the results presented in Table 1. The data in the first two lines demonstrate the expected characteristics of cooperative infection. Whether measured by the yield ratio (the fraction of cells that yielded at least one progeny phage) or by the modification ratio, several percent of the restricting cells were successfully infected by the unmodified phage if protein synthesis was not inhibited during the first few minutes of infection. Recombination frequencies were markedly enhanced when a restricting host was infected with T1.0 at a high MOI (compare the frequencies with those given for T1.P in Table 1 or with control data in reference 7). Data in the next two lines show the effect of CAM added 8 min before infection and removed 5 min after infection. Consistent with earlier results (10), the modification ratio was markedly reduced in the presence of CAM. The yield ratio was similarly reduced, indicating that CAM was actually affecting the success of the infection rather than the efficiency of modification. The burst size of successfully infected cells was, if anything, slightly larger than that of cooperatively infected cells. Finally, the recombination frequency was very markedly reduced, being lower, in fact, than in ordinary infection. This was true for both of the map intervals considered in these crosses and was most emphatic for the double-recombinant class, shown in the rightmost column.

The lower portion of Table 1 shows results of control experiments done with T1.P. As might

TABLE 1. *Effect of inhibition of protein synthesis on recombination in the presence and absence of restriction: crosses of modified and of unmodified T1 Sb Hr Kn⁺ × T1 Sb⁺ Hr⁺ Kn in E. coli B(P1), with and without CAM*

Phage	CAM	MOI	Yield ratio ^a (10 ⁻²)	Modifica- tion ratio ^b (10 ⁻²)	Phage yield/pro- ductively in- fected cell	Total no. of plaques scored	Recombinant progeny as a propor- tion of total progeny		
							Sb + Kn and + Hr +	Sb Hr Kn and + + +	Sb + + and + Hr Kn
T1.0	Absent	23	9.0	8.5	4.0	1,631	0.14	0.25	0.13
		23	12	7.5	5.7	2,131	0.19	0.23	0.11
	Present	23	0.39	0.39	10.8	1,670	0.029	0.058	0.004
		22	0.48	0.25	10.7	1,540	0.029	0.061	0.006
T1.P	Absent	21	ND ^c	ND	6.3	1,617	0.056	0.13	0.023
		20			10.0	1,690	0.077	0.15	0.025
	Present	21	ND	ND	9.0	2,397	0.070	0.12	0.017
		20			18.3	1,049	0.090	0.12	0.025

^a Fraction of infected cells that yielded an infectious center on a lawn of *E. coli* B cells.

^b Fraction of infected cells that yielded a T1.P particle as measured by infectious centers on a lawn of a P1-lysogenic strain, *E. coli* B(P1).

^c ND, Not done.

be expected, the temporary presence of CAM had no detectable effect on the outcome of the infection.

In general, in the absence of CAM, T1.0 recombination under restricting conditions (cooperative recombination) was higher than T1 recombination under standard conditions (when no restriction was present). However, the presence of CAM early during infection decreased recombination under restricting conditions so that it was lower than the frequency of recombination seen under standard conditions. These results further point to the special importance of protein synthesis during the first few minutes after infection of a restricting host by T1.0.

Effect of recombination defects in the host on marker rescue. As noted above, the phenomenon of cooperative infection requires certain physiological conditions for a substantial proportion of the restricting cells to be productively infected (6, 10). Recombination defects in the host have no effect on cooperative infection or on the accompanying increase in recombination (7). In contrast, in marker rescue crosses, where one of the input phages is modified and the other is unmodified, no special conditions are required for the rescue of markers from T1.0 by coinfection or superinfection with T1.P (2, 4). Because of the different physiological requirements for cooperative infection and its accompanying recombination on the one hand and marker rescue on the other, we considered the possibility that different recombination pathways were involved in the two phenomena. Table 2 presents the results of experiments designed to determine whether recombination defects in the host would affect marker rescue. In

no case was a recombination defect in the host associated with a deficiency in marker rescue. The *recA* (P1CM) host did produce more progeny with the complete genotype of the T1.0 parent than did the other strains tested here. The occurrence of progeny with two markers from the unmodified parent was also somewhat increased, but these phage classes still represented only a minor proportion of the total recovery of markers from the T1.0 parent. This increased leakage of markers can be attributed to the reduced restriction capacity of this host, which we have noticed before (7). Nevertheless, it is clear that the host's *recA* and *recB,C* functions were not required for marker rescue, just as they were dispensible in ordinary crosses and in the recombination accompanying cooperative infection (7).

Effects of inhibition of protein synthesis on rescue by modified phage of markers from unmodified phage. The presence of CAM during the first 5 min of infection almost completely blocked marker rescue (Table 3). Thus, in marker rescue crosses as in cooperative crosses (Table 2; 10), the functional survival of T1.0 as measured by its ability to participate in recombination was adversely affected by protein synthesis inhibition during the first 5 min of infection.

The fact that marker rescue was not detectably affected when T1.0 and T1.P phages were added separately (2, 4) allowed us to ask whether there is a requirement for either of the two phages to infect under conditions that allow protein synthesis in order for marker rescue to occur. Table 4 shows the effect of dividing the input of phages and of blocking protein synthesis

TABLE 2. Rescue of markers from T1.0 Sb Hr Kn⁺ by coinfection with T1.P Sb⁺ Hr⁺ Kn in hosts of different recombination proficiencies

Host genotype	Total no. of progeny counted	Progeny with markers from the restricted phage							Total rescue frequency		
		Sb only	Hr only	Kn ⁺ only	Sb and Hr	Sb and Kn ⁺	Hr and Kn ⁺	Sb, Hr, and Kn ⁺ ^a	Sb	Hr	Kn ⁺
<i>rec</i> ⁺ (P1CM)	1,484	50	92	107	0	3	8	6	0.036	0.069	0.080
	3,148	89	180	247	5	11	32	12	0.053	0.071	0.092
<i>recA</i> (P1CM)	3,900	178	234	291	4	32	43	505	0.055	0.072	0.094
	1,108	13	83	77	0	12	13	190	0.023	0.086	0.092
<i>recB</i> (P1CM)	3,373	102	153	268	0	12	21	46	0.034	0.052	0.089
	1,897	33	69	109	0	0	10	20	0.017	0.041	0.063
<i>recA recB</i> (P1CM)	4,207	120	156	358	0	8	12	18	0.030	0.040	0.090
	1,697	48	68	148	2	7	13	3	0.036	0.049	0.099

^a Assumed to represent the low-level production of the unmodified parental phage by nonrestricting cells in the population, rather than being rescue products. These progeny are not included in the calculation of the total rescue frequency.

before infection by either of the parental phages. Looking first at the cases in which infection by T1.0 preceded that by T1.P, it is clear that the addition of CAM between the two infections did not prevent rescue. Therefore, T1.0 could stimulate whatever protein synthesis was required within 3 min after infection for marker rescue to occur. If protein synthesis was allowed for 3 min after T1.P infection, then blocking protein synthesis before adding T1.0 also did not affect the rescue frequency. Therefore, the protein(s) necessary for the rescue of markers from T1.0 was not synthesized in response to the events of restriction, but appeared to be made early after either T1.P or T1.0 infection.

DISCUSSION

Immediately after infection by unmodified phage in a restricting host, protein synthesis is required to ensure the survival of restricted DNA in a form able to participate in recombination. The unmodified DNA that has thus been preserved can undergo the events of recombi-

nation at later times from 5 min (Table 4) up to 1 h (2).

On the basis of our data, some hypotheses about the role of the required protein(s) are made less plausible. (i) Marker rescue recombination, like cooperative recombination (7), did not depend on the host's recombination system or on the host's inducible repair responses, both of which were shown to be under *recA* control (Table 2). Therefore, inhibition of protein synthesis cannot affect survival of T1.0 by blocking the inducible responses of the host under *recA* control (5, 14). (ii) Expression of genes during the first few minutes of infection does not involve the synthesis of a protein that antagonizes the restriction system itself. This is indicated by the fact that the fraction of unmodified phage that was found in the final burst was unaltered whether protein synthesis was blocked before simultaneous infection (Table 3) or whether T1.P was allowed to express early functions before the addition of T1.0 (Table 4). (iii) In all cases examined, the events of restriction oc-

TABLE 3. Effect of protein synthesis inhibition on the rescue of markers from T1.0 Sb Hr Kn⁺ by coinfection of B(P1) with T1.P Sb⁺ Hr⁺ Kn

CAM	Total no. of progeny counted	Progeny with markers from the unmodified phage							Total rescue frequency		
		Sb only	Hr only	Kn ⁺ only	Sb and Hr	Sb and Kn ⁺	Hr and Kn ⁺	Sb, Hr, and Kn ⁺ ^a	Sb	Hr	Kn ⁺
Absent	3,373	120	140	239	7	17	22	19	0.043	0.050	0.082
	2,808	92	138	159	6	8	19	11	0.038	0.058	0.066
Present	3,023	1	2	25	0	0	1	3	0.0003	0.001	0.009
	2,111	2	2	25	0	0	0	3	0.001	0.001	0.01

^a See Table 2.

TABLE 4. Rescue of markers from T1.0 Sb Hr Kn⁺ by either prior or subsequent infection with T1.P Sb⁺ Hr⁺ Kn and the effect of adding CAM before the second phage was added

Phage at 0 min	CAM at 3 min	Phage at 5 min	Total no. of plaques counted	Progeny with markers from the unmodified phage							Total rescue frequency		
				Sb only	Hr only	Kn ⁺ only	Sb and Hr	Sb and Kn ⁺	Hr and Kn ⁺	Sb, Hr, and Kn ⁺ ^a	Sb	Hr	Kn ⁺
Unmodified	Absent	Modified	1,578	43	46	78	0	0	13	45	0.027	0.037	0.058
			1,990	82	170	159	14	22	44	423	0.059	0.11	0.11
Unmodified	Present	Modified	3,267	84	90	151	0	3	13	49	0.027	0.032	0.051
			2,572	117	143	164	2	16	37	177	0.052	0.071	0.084
Modified	Absent	Unmodified	2,533	36	35	70	0	1	2	19	0.015	0.015	0.029
			2,257	22	26	50	0	0	10	10	0.010	0.016	0.027
Modified	Present	Unmodified	2,285	78	77	121	0	4	14	18	0.036	0.040	0.061
			2,090	61	71	93	7	1	4	8	0.033	0.039	0.061

^a See Table 2.

curred within the first few minutes after injection of unmodified DNA (1). In the case of phage T1, it has been shown that DNA restriction and degradation to oligonucleotides is completed within the first 5 min after infection (10). The presence of CAM early during T1.0 infection has little effect on the degradation of the infecting DNA: approximately 25% of the incoming unmodified DNA is not degraded to acid-soluble fragments regardless of whether CAM is present (10). The size distribution, physical state, and molecular form of this remaining DNA are unknown. Markers from unmodified DNA were available for marker rescue recombination even if modified phage was added 5 min after infection by unmodified phage (Table 4). By this time, all events of restriction are expected to be completed (10). Christensen has shown that markers on unmodified phage are available for recombination even if superinfection by modified phage occurs 1 h after infection by unmodified phage (2). Therefore, the immediate need for protein synthesis cannot reflect an urgent need for recombination proteins.

The hypothetical protein(s) can be envisaged to prevent the degradation of restricted recombinogenic DNA: (i) by antagonizing a host nuclease, such as the *gam* gene product of phage lambda (11), (ii) by binding to restricted DNA and thus preventing nucleolytic degradation (9), or (iii) by maintaining the restricted DNA in a recombinogenic form by aiding in a step such as membrane attachment, thus sequestering the DNA from the action of host nucleases. The demonstration by Wagner et al. (13) and Schweiger et al. (12) that T1 infection causes structural and functional alterations of the host cell membrane makes the last hypothesis more tenable.

ACKNOWLEDGMENTS

We thank David Figurski and Thomas V. Potts for helpful

discussions during the course of this work.

Support for this work was provided by Public Health Service research grant AI-02781 and training grants DE 00003 and GM 00592, all from the National Institutes of Health.

LITERATURE CITED

1. Arber, W. 1974. DNA modification and restriction. *Prog. Nucleic Acid Res. Mol. Biol.* 14:1-37.
2. Christensen, J. R. 1962. The fate of genes from restricted T1 in lysogenic *Shigella dysenteriae*, Sh(P1). *Virology* 16:133-139.
3. Christensen, J. R. 1974. Complementation by restricted phage T1. *J. Virol.* 14:1411-1418.
4. Drexler, H., and J. R. Christensen. 1961. Genetic crosses between restricted and unrestricted T1 in lysogenic and nonlysogenic hosts. *Virology* 13:31-39.
5. Eisenstark, A. 1977. Genetic recombination in bacteria. *Annu. Rev. Genet.* 11:369-396.
6. Freshman, M., S. A. Wannag, and J. R. Christensen. 1968. Cooperative infection of P1-lysogenic bacteria by restricted phage T1. *Virology* 35:427-438.
7. Kotval, J. S., T. V. Potts, and J. R. Christensen. 1979. Recombination in bacteriophage T1 in the presence of host restriction. *J. Virol.* 31:17-24.
8. Lederberg, S. 1957. Suppression of the multiplication of heterologous bacteriophage in lysogenic bacteria. *Virology* 3:496-513.
9. Molineaux, I., and M. L. Gelfert. 1975. Properties of the *Escherichia coli* DNA-binding (unwinding) protein interaction with nucleolytic enzymes and DNA. *J. Mol. Biol.* 98:811-825.
10. Potts, T. V., and J. R. Christensen. 1974. Physiological study of cooperative infection by restricted bacteriophage T1. *J. Virol.* 14:1319-1325.
11. Sakaki, Y., A. E. Karu, S. Linn, and H. Echols. 1973. Purification and properties of the *gam*-protein specified by bacteriophage lambda: an inhibitor of the host RecBC recombination enzyme. *Proc. Natl. Acad. Sci. U.S.A.* 70:2215-2219.
12. Schweiger, M., E. F. Wagner, M. Hirschkauffmann, H. Ponta, and P. Herrlich. 1978. Biochemistry of development of *E. coli* viruses T7 and T1, p. 171-186. In B. F. C. Clark, H. Klenow, and J. Zeuthen (ed.), *Gene expression*. Pergamon Press, Inc., New York.
13. Wagner, E. F., H. Ponta, and M. Schewiger. 1980. Development of *Escherichia coli* virus T1. The role of the proton motive force. *J. Biol. Chem.* 255:534-539.
14. Witkin, E. M. 1976. Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. *Bacteriol. Rev.* 40:869-907.