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The *ocr*$^+$ Gene Function of Bacteriophages T3 and T7 Counteracts the *Salmonella typhimurium* DNA Restriction Systems SA and SB

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In host cells containing the *Salmonella typhimurium* DNA restriction-modification systems SA$^+$ and SB$^+$, replication of the *ocr*$^+$ bacteriophages T3 and T7 is not impaired. However, *ocr* (gene 0.3) mutants of these phages are susceptible to DNA restriction and modification by the SA$^+$ and SB$^+$ systems.

Salmonella typhimurium strains code for certain DNA restriction and modification enzymes, of which the systems SA and SB, as well as LT, are best known (3, 4, 12). Up to now these enzymes have not been isolated and characterized biochemically, but several data indicate that they are complex type I restriction endonucleases (combined with DNA-adenine methylases [3–6, 12; N. E. Murray, J. A. Gough, B. Suri, and T. A. Bickle, Eur. Mol. Biol. Org. J., in press]).

When cells of *Escherichia coli* B or K are infected by bacteriophage T3 or T7, the phage-coded *ocr*$^+$ protein (gp 0.3) blocks the type I restriction systems of the host cells and thereby prevents both DNA cleavage and methylation (for a review, see reference 7). The *ocr*$^+$ gene function is also capable of inhibiting the endonucleolytic and methylating activities of type III restriction enzymes (e.g., *Eco*P1 [9]); however, type II endonucleases are affected neither in vivo (D. H. Krüger et al., manuscript in preparation) nor by the purified *ocr*$^+$ protein in vitro (10; Krüger et al., in preparation).

We have now examined the effect of *ocr*$^+$ on the action of the SA and SB DNA host specificity systems of *S. typhimurium*. Since T3 and T7 generally do not adsorb to *Salmonella* cells (2), we used the following *E. coli* strains into which the SA, SB, or both host specificity genes had been introduced by Van Pel and Colson (12) through conjugation with *S. typhimurium Hfr* cells: strain 4614 (WA3703) (SA$^+$ SB$^+$), strain 4619 (WA3704) (SA$^+$ SB$^-$), strain 4620 (WA3705) (SA$^-$ SB$^+$), and strain 4621 (WA3706) (SA$^-$ SB$^-$).

Table 1 shows the growth data for T3 and T7 *ocr*$^+$ and *ocr* derivatives in the various hosts. Whereas the replication of *ocr*$^+$ strains was not inhibited by the SA and SB systems, SA$^+$ strains reduced the efficiency of plating (EOP) of the phage T3 *ocr* mutant T3/R7 fivefold, and SB$^+$ restricted the phage T7 *ocr* mutant T7/D111 30-fold. Phage adsorption was not affected (data not shown).

The *ocr* phages subject to restriction could also be host-specifically modified by the SA$^+$ and SB$^+$ systems (Table 2). It should be pointed

TABLE 1. Growth of T3 and T7 phage and their *ocr* mutants on host strains with *Salmonella* SA and SB DNA restriction systems

Phage[a]	Gene 0.3 function of phage[b]	Relative EOP[c] on *E. coli* strain:				
		WA921 (*Eco*K$^-$)	WA3703 (SA$^+$ SB$^+$)	WA3704 (SA$^+$ SB$^-$)	WA3705 (SA$^-$ SB$^+$)	WA3706 (SA$^-$ SB$^-$)
T3	*ocr*$^+$	1	1	1	1	1
T3*sam*$^-$	*ocr*$^+$	1	1	1	1	1
T3/R7	*ocr*	1	2×10^{-1}	2×10^{-1}	1	1
T7	*ocr*$^+$	1	1	1	1	1
T7/D111	*ocr*	1	3×10^{-2}	1	3×10^{-2}	1
Lambda (control)		1	3×10^{-6}	3×10^{-5}	3×10^{-4}	1

[a] Phage were grown on *E. coli* WA921 (*hsdR hsdM*).
[b] For the characterization of the phage derivatives used here, see reference 8.
[c] Relative EOP values (determined in parallel) are the ratios of titers on test indicator strains to titers on permissive strain WA921. All incubations were at 30°C (4).

TABLE 2. Host-dependent modification of *ocr* phage by *Salmonella* systems[a]

Phage (strain)	Relative EOP[b] on *E. coli* strain:				
	WA921 (*Eco*K$^-$)	WA3703 (SA$^+$ SB$^+$)	WA3704 (SA$^+$ SB$^-$)	WA3705 (SA$^-$ SB$^+$)	WA3706 (SA$^-$ SB$^-$)
T3/R7 (WA921)	1	2×10^{-1}	2×10^{-1}	1	1
T3/R7 (WA921, WA3704)	1	7×10^{-1}	7×10^{-1}	1	1
T3/R7 (WA921, WA3704, WA3706)	1	2×10^{-1}	2×10^{-1}	1	1
T7/D111 (WA921)	1	3×10^{-2}	1	3×10^{-2}	1
T7/D111 (WA921, WA3705)	1	6×10^{-1}	1	6×10^{-1}	1
T7/D111 (WA921, WA3705, WA3706)	1	3×10^{-2}	1	3×10^{-2}	1

[a] Single plaques grown on the indicated host strains were dispersed in phage buffer; suitable dilutions of phage were mixed with the respective host indicator cells and overlay agar and poured into petri dishes containing basal agar. All phage propagations were performed in nutrient broth medium at 30°C.

[b] See Table 1, footnote *c*.

out that host-modified *ocr* phages escaped restriction to an appreciable degree when replated on the same host; however, the EOP never reached unity (Table 2). (Incomplete modification of *ocr* phages has also been described for *Eco*B and *Eco*K; this could be the consequence of incomplete methylation of the phage DNA [8, 11].) The modification effect was fully reversible, i.e., it disappeared after passage over a restriction-negative host strain (Table 2).

To demonstrate the degradation of *ocr* phage DNA in the restricting host cells, we labeled phage T7/D111 with [^{3}H]thymidine during propagation in a modification-negative host strain and used the purified phage to infect *E. coli* WA3705 (SB$^+$). The increase of acid-soluble radioactivity in the restricting host compared with that in appropriate controls showed that DNA restriction of *ocr* phage was taking place (Fig. 1).

Brunovskis and Burns (2) reported on the DNA restriction of wild-type T7 in *S. typhimurium* 409 cells. If these results prove to be reproducible (they are difficult to interpret because impaired phage adsorption and intracellular restriction are not kept apart, and the differentiation between "mutated" and "modified" phage is insufficient), they represent a restriction system that is independent of SA and SB and attacks T7 DNA in vivo.

Our data show that the *ocr*$^+$ gene function of phages T3 and T7 is capable of protecting phage DNA from the effects of the *S. typhimurium* host specificity systems SA and SB. The *ocr* mutants are restricted and modified in vivo. The role of *ocr*$^+$-mediated protection is most obvious in SB$^+$ cells infected with phage T7 compared with its *ocr* mutant T7/D111. The T7 genome apparently does not contain recognition sites for the SA system, since T7/D111 was not restricted in SA$^+$ cells. With the phage T3 *ocr* mutant T3/R7, there was restriction by the SA$^+$ but not by the SB$^+$ system. The closely related phages T3 and T7 seem to possess different numbers of SA- and SB-specific recognition sites in their genomes. Such differences between T3 and T7 DNA in the number and localization of recognition sites for restriction enzymes have also been reported for other endonucleases, e.g., *Ava*I, *Hin*dIII, *Kpn*I,

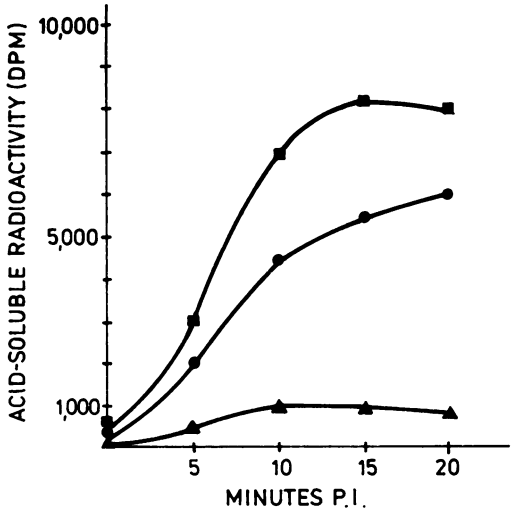

FIG. 1. DNA breakdown of [^{3}H]thymidine-labeled phage T7/D111 (*ocr*) in *E. coli* WA3705 (SB$^+$) cells. [^{3}H]DNA-labeled phage were grown as described (8). Logarithmically dividing cells were concentrated to a titer of 10^9 cells per ml, infected with 0.1 phage per cell, and incubated at 30°C. At the indicated times, 0.9 ml of the infected mixture was added to 0.1 ml of ice-cold 50% trichloroacetic acid. After 30 min in an ice bath, the samples were spun in an Eppendorf centrifuge, and the acid-soluble radioactivity contained in the supernatant was counted. The total radioactivity of phage DNA in a 0.9-ml sample was 18,000 dpm; nearly 65% of the phage (12,000 dpm) adsorbed to the cells after 5 min. The specific radioactivity of the phage was 2×10^{-4} dpm/PFU. Infection of *E. coli* strains WA921 (*hsdR hsdM*) (▲), WA3705 (SB$^+$) (●), and WA960 (*Eco*B$^+$) (■) with phage T7/D111. P.I., Postinfection.

*Mbo*I, *Xba*I (1), and *Eco*RV (D. H. Krüger et al., in preparation).

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