The *ocr* Gene Function of Bacterial Viruses T3 and T7 Prevents Host-controlled Modification

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**SUMMARY**

On pre-infection of the host *Escherichia coli* B with u.v.-inactivated T3 or T7 phage able to express their early genes (like o·3), B-specific modification of super-infecting, successfully multiplying viruses does not take place. The *ocr* gene function (gene o·3) of T3 and T7 not only prevents host-specific DNA restriction but also modification, probably by inhibiting the same late step in the interaction between the restriction enzyme and DNA.

The DNA of bacteriophages T3 and T7 is not subject to *in vivo* restriction by the common *E. coli* strains. Phages T3 and T7 possess an early gene function which overcomes classical restriction (*ocr*), in addition to the *sam* gene function of T3 (*S*-adenosylmethionine cleaving activity). Both *ocr* and *sam* are located in the gene o·3 region (Studier, 1975; Studier & Movva, 1976; Krüger *et al*. 1977c). The *ocr* gene protects the virus DNA and other foreign DNAs against intracellular cleavage by type I restriction endonucleases (Krüger *et al*. 1977b, c, 1978). Because the type I enzymes (*Eco*B, *Eco*K, *Eco*P1) are complex enzymes with endonucleolytic and DNA-modifying (methylating) activity (Haberman *et al*. 1972; Vovis *et al*. 1974), we have investigated whether *ocr* also prevents host-specific DNA modification.

The basic idea of the experiment was as follows: phage which normally undergo host-specific modification and restriction are not restricted by the host on which they were previously propagated since they have acquired the specific modification of the second host. However, if one prevents modification during the first growth cycle of the phage it is restricted during the next passage on the homologous host. In this way we can see whether pre-infection of *E. coli* B with u.v.-inactivated T3 or T7 still able to express their early genes turns off the modification capacity of the cells, i.e. whether a superinfecting test-phage is modified or not. If the progeny of the superinfecting phage carries the B-specific modification they are not restricted when plated on *E. coli* B, but if modification of the superinfecting phage is prevented by the pre-infecting virus, the efficiency of plating (e.o.p.) of the test-phage progeny on *E. coli* B will be reduced.

The virus derivatives and their origin are described by Krüger *et al*. (1977c); their early gene functions are shown in Table 1. The host strains *E. coli* 960 r*+*m*−* and *E. coli* 921 r*−*m*+ (Wood, 1966) are gifts from W. Arber (Basle) and were designated as *E. coli* B or O, respectively. The phages for pre-infection were purified by ultracentrifugation, resuspended in phage buffer and u.v.-inactivated by 4 to 5 orders of magnitude. Following infection, these u.v.-inactivated viruses can express their early genes to the same extent as non-irradiated phage (Gold *et al*. 1966; Hausmann & Härlé, 1971; Krüger *et al*. 1978). A culture of *E. coli* B in the logarithmic growth phase (2 × 10⁸/ml) was infected with u.v.-phage [multiplicity of infection (m.o.i.) 10] and after 3 min was superinfected with the test phage R7 or D111 (m.o.i. 1). After another 5 min for adsorption, the infected cells were separated from free virus by low speed centrifugation at 4 °C and resuspended in fresh broth. A
Table 1. Propagation of R7 or D111 test phages on E. coli B cells pre-infected with ocr+ or ocr− phages

<table>
<thead>
<tr>
<th>Test phage</th>
<th>Pre-infecting phage</th>
<th>Early gene functions of pre-infecting phage</th>
<th>Burst size of test phage (determined on E. coli O)</th>
<th>Restriction coefficient*</th>
<th>Modification of test-phage progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>R7.B</td>
<td>—</td>
<td>—</td>
<td>60</td>
<td>1.0</td>
<td>R7.B</td>
</tr>
<tr>
<td>R7.B</td>
<td>u.v.-T3</td>
<td>ocr+sam+</td>
<td>107</td>
<td>0.03</td>
<td>R7.B</td>
</tr>
<tr>
<td>R7.B</td>
<td>u.v.-T3sam−</td>
<td>ocr+sam−</td>
<td>65</td>
<td>0.09</td>
<td>R7.B</td>
</tr>
<tr>
<td>R7.B</td>
<td>u.v.-T7</td>
<td>ocr+sam−</td>
<td>110</td>
<td>0.02</td>
<td>R7.B</td>
</tr>
<tr>
<td>R7.B</td>
<td>u.v.-R7</td>
<td>ocr−sam−</td>
<td>102</td>
<td>1.0</td>
<td>R7.B</td>
</tr>
<tr>
<td>R7.B</td>
<td>u.v.-D111</td>
<td>ocr−sam−</td>
<td>50</td>
<td>0.0</td>
<td>R7.B</td>
</tr>
<tr>
<td>D111.B</td>
<td>—</td>
<td>—</td>
<td>82</td>
<td>1.0</td>
<td>D111.B</td>
</tr>
<tr>
<td>D111.B</td>
<td>u.v.-T3</td>
<td>ocr+sam+</td>
<td>100</td>
<td>0.04</td>
<td>D111.B</td>
</tr>
<tr>
<td>D111.B</td>
<td>u.v.-T3sam−</td>
<td>ocr+sam−</td>
<td>93</td>
<td>0.1</td>
<td>D111.B</td>
</tr>
<tr>
<td>D111.B</td>
<td>u.v.-T7</td>
<td>ocr+sam−</td>
<td>105</td>
<td>0.02</td>
<td>D111.B</td>
</tr>
<tr>
<td>D111.B</td>
<td>u.v.-R7</td>
<td>ocr−sam−</td>
<td>54</td>
<td>1.0</td>
<td>D111.B</td>
</tr>
<tr>
<td>D111.B</td>
<td>u.v.-D111</td>
<td>ocr−sam−</td>
<td>85</td>
<td>1.0</td>
<td>D111.B</td>
</tr>
</tbody>
</table>

* The restriction coefficient expresses the relative e.o.p. of progeny phage on E. coli B compared with E. coli O. The e.o.p. of the test-phage burst without pre-infection of the cells was taken to be 1.0, and all other e.o.p.s are expressed relative to this value. [Absolutely, the e.o.p. also of B-modified phage on E. coli B are a little lower than on E. coli O, due to their reduced adsorption on B cells (Krüger et al. 1977a, c).]

sample was immediately plated on E. coli O. The culture was incubated for another 25 min at 37 °C and the lysate was plated in parallel on E. coli O and B.

To study the influence of the ocr gene function on the modification of a phage which normally undergoes modification and restriction in the E. coli B – K – O system of hosts, lambda could not be used as the test phage since pre-infection of E. coli cells with T3 prevents the multiplication of lambda (data not shown here). Therefore the T3 mutant R7 and the T7 mutant D111 were used as test phage, which, like lambda, are modified and restricted in the E. coli B – K – O system (Studier, 1975; Studier & Movva, 1976; Krüger et al. 1977c). The results shown in Table 1 prove that, despite pre-infection with the different u.v.-irradiated T3 and T7 derivatives, the superinfecting test phage grows as well in terms of the burst size as without pre-infection. These results are in agreement with other superinfection experiments (Krüger et al. 1975, 1977c) and in contrast to conclusions of Hirsch-Kaufmann et al. (1976); a detailed discussion will be given elsewhere (D. H. Krüger, unpublished data).

Table 1 shows that the test phage R7 and D111 grown in E. coli B cells plate for practical purposes just as well on E. coli B as on E. coli O, i.e. they are B-modified. When the cells are pre-infected with the ocr− phage u.v.-R7 or u.v.-D111, modification of the test phage also takes place. In contrast to this, the progeny of test phage from E. coli B cells pre-infected with u.v.-T3, u.v.-T3sam−, or u.v.-T7, are restricted when plated on E. coli B. Thus, the B-specific modification of the test phage in E. coli B cells was prevented by the pre-infecting virus. Since this effect is not only achieved by u.v.-T3 (ocr+sam+) but also by u.v.-T3sam− and u.v.-T7 (both ocr+sam−), it is proved that, also in the absence of sam+, the ocr gene function can prevent host-specific modification of virus DNA. To make sure that non-classical modification and restriction, i.e. reversible changes in the adsorption values of the phage (Krüger et al. 1977a), are not interfering, adsorption values of the viruses were determined and different adsorption properties of the differently modified test phage could be excluded as the cause of the observed restriction (data not shown).
The results presented here clearly show that the \textit{ocr} gene function of T3 and T7 prevents host-controlled modification of phage. We conclude that \textit{ocr} not only blocks the endonucleolytic (Krüger \textit{et al.} 1977\textit{b, c}) but also the methylating activity of type I DNA restriction enzymes. At the same time the function of the gene o-3 protein of phage T7 postulated already by Studier (1975) to prevent restriction as well as modification, was practically confirmed.

Since \textit{ocr} only prevents modification methylation but not the overall methylation of the DNA, it becomes understandable that T7 DNA carries methyl groups. However, by comparison with \textit{E. coli} these 14 methyl groups per T7 genome (Günthert, 1975) are less than would be expected on the basis of the GC content and the molecular weight of T7 DNA. The intracellular prevention of DNA modification by the \textit{ocr} gene also explains the result of Eskin \textit{et al.} (1973) that the DNA of T7 wild-type virus (\textit{ocr}+) grown in \textit{E. coli} B is susceptible \textit{in vitro} to the cleavage by purified restriction endonuclease R.EcoB. In contrast to T7 DNA, T3 DNA is not methylated at all since the \textit{sam} gene function destroys the intracellular methyl donor S-adenosylmethionine (Gold \textit{et al.} 1966).

A few years ago attempts were made to prevent host-specific modification of super-infecting test phage by pre-infecting cells with u.v.-T3 (Klein, 1965; Hirsch-Kauffman & Sauerbier, 1968; Grasso & Paigen, 1969). In the light of our results, the conclusions drawn by these authors, according to which the prevention of modification is due to the \textit{sam} gene function, must be extended, i.e. the presence of the \textit{ocr} gene function alone is sufficient to achieve the same effect.

The \textit{ocr} gene function inhibits a later step in the interaction between cellular type I restriction enzymes and DNA than the \textit{sam} gene function (Krüger \textit{et al.} 1977\textit{b, c} and unpublished results). The data presented in this paper indicate that \textit{ocr} not only prevents the cleavage but also the DNA modification reaction of the enzyme. \textit{In vitro} studies on the influence of \textit{ocr} on the DNA methylation patterns are in progress.

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\textbf{REFERENCES}


Short communications


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