INFECTIVITY OF VARIOUS FORMS OF BACTERIOPHAGE T7 DNA

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INTRODUCTION

Whereas the DNA of most bacteria, phage and plasmids replicates via circular intermediates, the DNA of E.coli phage T7 replicates as a linear duplex during the first round of replication (1; 2) and also as a linear polymer during the late stages of intracellular phage development. The DNA isolated from mature T7 phages is a duplex of 25×10^6 daltons molecular weight, is non-permuted and has terminal redundancies each being approx. 1% of the total length. Transfection of lysozyme-EDTA spheroplasts prepared from E.coli has been performed with native and single-stranded T7 DNA (3; 4; 5). We have studied the infectivity of various forms of T7 DNA in order to examine the effect of host enzymes of the DNA metabolism on the biological activity of this DNA and also as an approach to gain some insight into the structural requirements for T7 DNA replication.

MATERIALS AND METHODS

Bacterial and phage strains: T7 wildtype (W. Studier) was used throughout. Properties and sources of the E.coli strains employed for transfection experiments (AB 1157; AB 2470; BW 46; JC 7623; KL 197; HMS 83; BT 10265) have been reported (5). AB 1157 was the plating indicator.

Isolation of phage DNA: Unlabelled phage T7 was concentrated from crude lysates by differential centrifugation. DNA was isolated by threefold extraction with phenol followed by dialysis against 20 mM Tris-HCl, pH 7.5, 1 mM EDTA. λ DNA was isolated as reported (6).

Separation of T7 DNA strands: ^{3}H-thymidine-labelled T7 was prepared and purified as reported (5). Poly UG-CsCl gradient centrifugation (7) was used to separate l and r strands (5). Transfection was performed without removal of poly UG from the DNA samples.

Preparation of T7 DNA with single-stranded terminal redundancies: 3'OH single-stranded redundancies were produced by degrada-

tion of 1% total DNA per T7 molecule with purified λ exonuclease. Details of the procedures will be given elsewhere (8).

Preparation of spheroplasts and transfection: Spheroplasts were prepared by lysozyme-EDTA treatment and transfection was performed as reported (9). Infected spheroplasts were assayed according to Wackernagel (10). Details on incubation etc. have been published (5). The efficiency is the amount of infected spheroplasts per DNA molecule.

RESULTS

Infectivity of native and single-stranded T7 DNA in E.coli lacking different DNases: We examined the effect of two DNases of the host on the infectivity of T7 DNA, namely the recBC DNase and

TABLE 1

Infectivity of native T7 DNA in E.coli strains lacking recBC DNase and/or exonuclease I

Strain	RecBC DNase	Exo I	Efficiency*	Factor
AB 1157	+	+	30	1
AB 2470	-	+	1600	53.3
BW 46	+	-	8.4	1
JC 7623	-	-	690	82.1

*Infective centers per DNA molecule ($x10^8$)

exonuclease I. The former degrades native and denatured DNA in the presence of ATP (11), whereas the latter acts specifically on single strands (12). For the experiments we prepared spheroplasts from a set of four strains including wildtype and mutants defective for one or both enzymes (Table 1). Transfection with native DNA indicated (Table 1) that the infectivity of the DNA is significantly increased by the absence of the recBC DNase. This result is consistent with published data (4; 9; 10) and suggests that more than 90% of native linear DNA molecules are destroyed in the spheroplasts by the recBC DNase. Absence of exonuclease I did not stimulate the infectivity of native DNA as expected from the substrate specificity of this enzyme. When the four strains were infected with separated l and r strands of T7 (Table 2), infectivity was increased about threefold in strains deficient for recBC DNase. A more pronounced stimulation (10 fold) has been observed with heat denatured DNA (5). It appears, that the recBC DNase does not attack

TABLE 2

Infectivity of separated l and r strands of T7 DNA in E.coli strains lacking recBC DNase and/or exonuclease I

Strain	RecBC DNase	Exo I	Efficiency ($x10^7$)* l strand	Efficiency ($x10^7$)* r strand	l/r
AB 1157	+	+	4.5	2.3	2.0
AB 2470	-	+	14.7	7.5	2.0
BW 46	+	-	2.4	1.4	1.7
JC 7623	-	-	7.5	4.0	1.9

*Infective centers per DNA molecule

single strands as readily as native DNA in vivo. This observation supports earlier results (4) and parallels the preference of the enzyme for native DNA in vitro (11). Unexpectedly, the lack of exonuclease I did not stimulate single strand transfection. Possibly the infecting molecules do not enter the cellular compartment where exonuclease I is active or the properties of this enzyme in vivo differ from what has been observed in vitro. Table 2 also shows that l strands are twice as infective as r strands. The reason for this effect is unknown. Transfection with separated strands gave linear DNA concentration curves, which is in contrast to heat denatured DNA (5).

Infectivity of single-stranded T7 DNA in E.coli lacking various DNA polymerases: The infectivity of a single l or r strand of T7 DNA raises the question as to which of the host DNA polymerases converts the single strand into a transcribable duplex that can replicate. In order to answer this question we have transfected a series of E.coli mutants lacking one or several DNA polymerases with native DNA from T7 and λ and with separated strands of T7 DNA. The results (Table 3) show that native T7 DNA is infective even in strain BT 10265 at 42°, when no host DNA polymerase is active. This is consistent with the fact that T7 codes for its own replication machinerie (s. 13). Under the same conditions neither λ DNA nor T7 l strand are infective. It is known that λ DNA replication depends on a functional DNA polymerase III (14) and so is the infectivity of T7 l strand. An identical result was obtained with the r strand (not shown). These data suggest that E.coli DNA polymerase III supplies the complementary strand to an infecting single strand of T7 DNA.

TABLE 3

Infectivity of native T7 and λ DNA and T7 1 strand in E.coli strains defective for different DNA polymerases

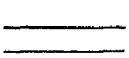
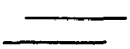
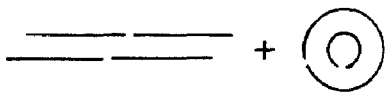
Strain	Relevant phenotype	Relative infectivity ($x10^8$)* of native T7 DNA	native λ DNA	T7 1 strand
AB 1157	Pol^+	30	5000	45
KL 197	Pol I^-	14	16	9
HMS 83	Pol $I^-;II^-$	38	5	138
BT 10265	Pol $I^-;II^-$;IIIts 30°	5.3	11	3.5
"	Pol $I^-;II^-$;IIIts 42°	3.5	<0.02	<0.05

*Infective centers per DNA molecule

Infectivity of 3'OH-terminal single-stranded T7 DNA and DNA polymers: DNA synthesis by DNA polymerase III is dependent on a 3'OH primer which in vivo probably consists of RNA (15). When DNA polymerase III provides the complementary strand to an infecting T7 single strand, then at least one terminal RNA primer would remain

TABLE 4

Infectivity in E.coli rec^+ (AB 1157) and recB (AB 2470) of native T7 DNA, terminally single-stranded T7 DNA (1.3% per end) and a mixture of various polymers and circles made by thermal annealing of terminally single-stranded T7 DNA

DNA structure	Relative infectivity in rec^+	recB	recB/rec^+
	1*	128*	128
	4.7	131	28
	10	390	39

*The absolute efficiencies for transfection were $1.6x10^{-9}$ in rec^+ and $2.6x10^{-7}$ in recB.

in the duplex molecule. After removal of the primer by RNase H (16) a 5'-gap would result which could not be repaired by any known DNA polymerase. Nevertheless we observe plaque formation after single strand transfection at high efficiency. In order to study the effect of terminal 5'-gaps at both ends of native DNA molecules, T7 DNA was subjected to limited digestion with λ exonuclease, so that

all terminal redundant regions were converted into 3'OH single strands. Surprisingly, the biological activity of such molecules was unimpaired as compared with untreated native DNA (Table 4). Linear dependence of infectivity on DNA concentration indicated that one partially digested molecule suffices for the production of an infected center. When polymers (and possibly circular structures) were formed from the partially digested molecules by thermal annealing of the single-stranded terminal redundancies (at 11.2 ug DNA/ml in 0.6 M NaCl, 20 mM phosphate buffer, pH 7.5; slow cooling from 75° to 30° over a period of 4 h), the infectivity of this DNA preparation increased per unit length DNA (Table 4). Polymeric structures in the preparation were identified by neutral sucrose gradient centrifugation. The highest infectivity (more than 20 fold per unit length DNA as compared with untreated DNA) was associated with DNA sedimenting at a rate of trimers (8). Such polymers which are similar to possible replication intermediates of T7 (17), apparently provide some protection to internal genomes against exonuclease attack *in vivo*, an effect which is more prominent in wildtype cells than in *recB* cells.

DISCUSSION

Transfecting native and single-stranded T7 DNA is subject to biological inactivation in recipient cells by the *recBC* DNase, but not by exonuclease I. In normal phage infection an attack of the *recBC* DNase apparently does not occur, since the efficiency of plating of T7 is almost identical on wildtype and *recB* cells. Nevertheless the *recBC* DNase would possibly interfere with some steps in the phage development if T7 did not code for a specific function which inactivates the *recBC* DNase within 6 min after phage infection (18; 20; 21).

The infectivity of a single strand of T7 DNA appears to depend on a functional *E.coli* DNA polymerase III. As already mentioned, this polymerase may provide the complementary strand, but the resulting molecule would have a 5' terminal gap (position of the RNA primer) which cannot be filled by any known DNA polymerase. On the other hand, replication of linear duplex molecules requires complete terminal redundant regions of unique base sequence as suggested by the model proposed by J.D. Watson (19). Accordingly, single strands should not be infective, but they are. Even molecules with two "irrepairable" terminal gaps, produced by limited

digestion with λ exonuclease, display the same infectivity as fully native molecules. This observation raises the question as to the necessity of the terminal redundancies for intracellular T7 development. According to the model of Watson (19) the complete and native redundancies are essential for T7 DNA replication and maturation. The results presented here and some recent experiments (8) indicate that this is not necessarily the case. In these experiments it was shown that T7 DNA, from which the terminal redundant regions were completely removed by subsequent treatment with λ exonuclease and exonuclease I, were almost as infective as untreated DNA in cells lacking the recBC DNase. The molecular structure of the DNA of T7 phages emerging from spheroplasts infected with genomes without terminal redundancies is presently being studied. If it turned out that these molecules do not contain terminal redundancies, then the replication of T7 might proceed also along an alternate pathway to the one envisaged by Watson (19).

SUMMARY

The infectivity of various DNA structures prepared from T7 DNA has been studied in lysozyme-EDTA spheroplasts from E.coli mutants defective for different DNases and DNA polymerases. The experiments show that the biological activity of native T7 DNA is decreased to 1 - 2% by the recBC DNase, but is independent of host DNA polymerases in the cells. The infectivity of single strands of T7 DNA is not affected by exonuclease I, is reduced by the recBC DNase and depends on active DNA polymerase III in polAB cells. Native T7 DNA with 3' terminal single strands, produced by limited digestion with λ exonuclease, is fully infective. Formation of polymers from such molecules by thermal annealing increases the infectivity above the level observed with native, untreated DNA. The results are discussed with respect to the mode of T7 DNA replication.

ACKNOWLEDGEMENTS

We thank Miss R. Velmelage for expert technical assistance and the Deutsche Forschungsgemeinschaft for support.

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