NOTES

Variable Transforming Activity of Nonglucosylated and Fragmented T4 DNA

LORE' ANNE McNICOL

Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts 02111, and Department of Human Genetics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19174

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Nonglucosylated and fragmented T4 DNA shows a gene-specific variation in transformation efficiency.

Coliphage T4 DNA is normally glucosylated by phage-induced α - and β -glycosyl transferase (gt) enzymes (6). T4 DNA will not be glucosylated either if the phage lacks both gt enzymes (gt phage) (2) or if the phage is grown on a host deficient in the uridinediphosphate glucose substrate (T4 * phage) (4). T4 phage with nonglucosylated DNA is restricted during infection of most *Escherichia coli* strains (12). This host-restricting function (*rgl*) is present in spheroplasts and acts on DNA during entry, but not during subsequent intracellular development (11). During infection of an *rgl*⁺ cell, nonglucosylated DNA is converted to an acid-soluble form (1). Such DNA shows a reduced ability to synthesize early enzymes and does not synthesize phage DNA or late proteins (3).

Although nonglucosylated T4 DNA will act as a genetic donor in transformation assays (13), this communication will demonstrate that its transforming activity is markedly low. Figure 1 compares the transformation frequency (TF) of wild-type, T4 *, and gt phage DNA for the *r*₆₁ marker assayed in *E. coli* B, which is *rgl*⁺. The transformation efficiencies (TE) of these DNA preparations were 1.0, 0.43, and 0.026, respectively. T4 * phage have glucose levels which are intermediate to those of wild-type and gt phage, and T4 * glucosylation varies markedly in different preparations (8a). The TE of T4 * DNA preparations (when assayed under the conditions described for Fig. 1) varied from 0.15 to 0.5. In contrast to these results with T4 * DNA, transformation with gt DNA was much more reproducible. Four different gt mutants, whose DNA had no glucose detectable by im-

munochemical methods (8a) gave TE's for *r*₆₁ that ranged from 0.25 to 0.30.

The lowered TE of nonglucosylated DNA is gene specific and not peculiar to the *r*₆₁ marker. Transformation of two other *rIIA* mutations (*r*₇₇ and *rH88*) with gt DNA gave TE's of 0.024 and 0.030 (see Table 1).

The most striking feature of transformation with nonglucosylated DNA is the variation in TE seen for different genes. Table 1 shows that the TE varies from 1.0 for genes 40-42-43 to 0.007 for *rIIB*, which is barely a measurable value. In addition to the lowered TE, transformation with nonglucosylated DNA results in: inhibition of the yield of progeny phage, up to 10-fold; reduced capacity to stabilize spheroplasts (11); and an hour's delay in the time of appearance of transformed phage.

Table 1 demonstrates that nonglucosylated DNA has a higher TE when markers are assayed in spheroplasts of *Aerobacter aerogenes* which are semi-permissive for nonglucosylated T4 phage (A.C. Wais, Ph.D. thesis, Tufts University School of Medicine, 1970). (Unfortunately, the *E. coli rgl*⁻ strains form spheroplasts which are not competent for transformation.) The physiological features of transformation with nonglucosylated DNA were more normal in the *A. aerogenes* assays than in *E. coli* B. Thus, it appears that the reduced transforming activity is related to restriction of the nonglucosylated DNA. Physical studies are consistent with this suggestion. After 20 min of incubation in *rgl*⁺ spheroplasts, 50% of the ³²P in nonglucosylated DNA is converted to material soluble in trichloroacetic acid, whereas wild-type DNA

FIG. 1. Transformation of the r_{61} marker by wild-type, $T4^*$, and gt phage DNA in rgl^+ spheroplasts. Spheroplasts of *E. coli* $B rgl^+$ were incubated with denatured DNA for 15 min at 37°C, then infected with r_{61} helper phage. After incubation at 37°C for 3 h, the cells were lysed with chloroform and the TF (ratio of wild-type phage to total phage progeny) was determined by differential plating. The details of transformation and DNA preparation are described elsewhere (8, 14, 15). $T4^*$ were prepared by growing wild-type phage in *E. coli* W4597 (obtained from H. R. Revel) for one cycle (4). The specific transformation frequency (sp. TF) is the initial slope of the TF versus DNA concentration curve for wild-type DNA (17). The transformation efficiency (TE) is a comparison of the initial slopes of these curves for (experimental DNA/wild-type DNA) (17). It is not clear why the $T4^*$ DNA curve is nonlinear at concentration where wild-type DNA is linear, but this result has been observed repeatedly. The gt phage was $\alpha gt^{71} \beta gt^{27}$. The wild-type phage was $T4 BO_{r1}$. Wild-type DNA (○), $T4^*$ DNA (▲), gt DNA (□).

remains completely insoluble. In the semi-permissive spheroplasts there is less degradation of nonglucosylated DNA (20% becomes acid soluble after 20 min of incubation).

One hypothesis to explain the data in Table 1 is that $rIIB$ represents the physical site of the primary restriction event. Markers on either side of this region would then have an increasingly higher probability of escaping degradation and therefore transform with higher frequencies. However, it is known that glucosylated DNA which has been fragmented by sonication has a reduced transformation efficiency (16). An alternative mechanism of fragmentation also produces DNA with a lowered TE. Fig. 2a demonstrates that 3BU -substituted $T4$ DNA transforms as well as normal DNA. However, when this DNA is broken by blacklight irradiation

TABLE 1. Transformation efficiency of nonglucosylated DNA in *E. coli* $B rgl^+$ and *Aerobacter aerogenes* spheroplasts^a

Gene ^b	Marker	sp. TF ^a	TE of gt DNA	
			rgl^+	Semi-permissive ^c
40	$tsL84$	15×10^{-6}	1.0 (2) ^d	0.90 (3)
42	$tsLB3$	1.6×10^{-6}	0.82 (3)	
43	$tsG40$	4.3×10^{-6}	0.85 (2)	
^e	$eG59$	2.6×10^{-6}	1.0 (1)	
3	$tsA2$	6.8×10^{-6}	0.58 (5)	
5	$tsB49$	9.3×10^{-6}	0.96 (1)	0.93 (2)
12	$tsB1$	12×10^{-6}	1.2 (5)	
21	$tsN8$	9×10^{-6}	0.79 (1)	
30	$tsA80$	8×10^{-6}	0.13 (6)	
34	$tsA44$	6.7×10^{-6}	0.19 (2)	
37	$tsN52$	0.4×10^{-6}	0.35 (2)	0.75 (3)
$rIIB$	r_{73}^e	2.1×10^{-6}	0.007 (3)	
$rIIA$	$rH88^f$	3.1×10^{-6}	0.03 (7)	
	r_{61}^g	3×10^{-6}	0.026 (3)	
	r_{77}^g	1.2×10^{-6}	0.024 (3)	

^a See the legend to Figure 1 for the definition and measurement of TE and sp. TF. $T4D \alpha gt^{71} \beta gt^{27}$ was used as the source of gt DNA.

^b Listed in physical order.

^c The details of transformation with *A. aerogenes* spheroplasts are described elsewhere (15).

^d The figures in parentheses indicate the number of replicate determinations.

^e This marker is presumably a small deletion (17).

^f This marker is a large deletion (5).

^g These markers are revertible point mutations (5, 17).

FIG. 2. Transformation of $rH88$ by normal and 3BU -substituted wild-type $T4$ DNA. $T4 BO_{r1}$ phage with 3BU -substituted DNA were prepared as previously described (7) and kept scrupulously in the dark. $rH88$ helper phage were transformed in *A. aerogenes* spheroplasts with varying concentrations of (A) normal, (○), and 3BU -substituted (□) phage DNA, handled in the dark; and (B) normal (▲) and 3BU -substituted (*) DNA from phage irradiated in a water-cooled well with a G.E. blacklight bulb (maximal emission at 350 nm). These phage received 100 lethal hits, judged from the plot of log percent survivors versus time of irradiation.

TABLE 2. Transformation efficiency of fragmented T4 DNA

Gene ^b	Marker	sp. TF ^a	TE of fragmented DNA ^a		
			Sonically treated ^c	Super sonically treated ^d	Irradiated ⁶⁰ Co ^e
40	<i>tsL84</i>	15×10^{-6}	0.73 (4) ^f	0.45 (5)	0.81 (4)
43	<i>tsG40</i>	4.3×10^{-6}	0.65 (5)	0.50 (4)	
^g	<i>eG59</i>	2.6×10^{-6}	1.1 (2)		
3	<i>tsA2</i>	6.8×10^{-6}	1.1 (2)		0 (5)
5	<i>tsB49</i>	9.3×10^{-6}	1.0 (1)		
12	<i>tsB1</i>	12×10^{-6}	0.8 (3)		
<i>rII B</i>	<i>r₇₃^h</i>	2.1×10^{-6}	0.45 (2)		0 (5)
<i>rII A</i>	<i>rH88^h</i>	3.1×10^{-6}	0.28 (3)		0 (4)
	<i>rb50ⁱ</i>	3.9×10^{-6}	0.40 (2)		

^a See the legend to Fig. 1 for the definition and measurement of TE and sp. TF. All DNA was prepared from the wild type phage T4 BO⁺.

^b Listed in physical order.

^c DNA at a concentration of 50 μ g/ml in 0.01 M K-PO₄ buffer, pH 7.4, was treated in a Raytheon oscillator for 15 min at 4 C. This material had a molecular weight of 0.5×10^6 to 10^{-6} , judged by sucrose gradient sedimentation.

^d DNA at a concentration of 50 μ g/ml in 0.01 M KPO₄ buffer, pH 7.4, was fragmented with the 1-cm probe of a Branson sonicator at maximal emission for 30 min at 4 C. This material had a molecular weight of 10^4 , judged by sucrose gradient sedimentation.

^e See the legend to Fig. 2.

^f The figures in parentheses indicate the number of replicate determinations.

^g This marker is presumably a small deletion (17).

^h This marker is a large deletion (5).

ⁱ This marker is a missense mutation. (K. M. Carlson, Ph.D. thesis, University of Pennsylvania, Philadelphia, 1971).

tion (Z. Lorkewicz, personal communication) (Fig. 2b), it has a markedly reduced transformation efficiency. Table 2 contains compiled data for both types of fragmented DNA, assayed for different markers. They show a variation in TE which is similar to that observed with nonglucosylated T4 DNA (in Table 1). Thus, different T4 genes may possess an inherent variation in their ability to be transformed by short fragments of DNA, regardless of the fragmentation mechanism.

The variation in TE reported in Tables 1 and 2 resembles the variation in transforming activity observed with DNA synthesized after infection with T4 incomplete chromosomes (9). It is not clear what these experiments have in common besides the fact that the donor DNA is smaller than mature phage DNA. A similar marker variation has been reported in the frequency with which incomplete T4 chromosomes transfer their markers to progeny when crossed with a complete chromosome (10). The variations in these four experimental systems do not correspond particularly well to the recombinational "hot spots" which have been described by Womack (15), although the *rII* region shows especially low activity in all cases. Some step in the transformation process must vary systematically with the size of the donor

fragment. But until more is known of the molecular mechanism of transformation, the basis of these variations will remain obscure.

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