A MUTANT OF BACTERIOPHAGE T4 DEFECTIVE IN α-GLUCOSYL TRANSFERASE

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After infection of E. coli, bacteriophage T4 induces the synthesis of two glucosyl transferases which catalyze the addition of monoglucosyl residues, in either α- or β-configuration, to all the hydroxymethyl cytosine (HMC) groups of T4 DNA (Kornberg et al., 1961). The present report deals with the isolation of a mutant of T4 defective in its ability to induce α-glucosyl transferase in phage infected cells of E. coli.

In the course of studies on the nature of phage DNA synthesized by various T4 amber mutants, the α-glucosyl transferaseless mutation (T4αgt) was found to be present as a second mutation in T4 amH39, a T4 derivative with an amber mutation in gene 30. AmH39 was originally classified DO, that is, defective in the synthesis of DNA. Recent studies (Bolle et al., in press), however, have shown that small amounts of DNA are made in cells of E. coli B infected with amH39: DNA synthesis begins at the normal time but stops shortly thereafter. These new results have been confirmed in this laboratory. In addition, it has been found that the amH39 DNA synthesized in E. coli B is not stable: radioactive thymidine incorporated into high molecular weight DNA later becomes acid-soluble. Wiberg, (1966), has shown that host DNA is not degraded in E. coli B cells infected with T4 mutants defective in genes 46 or

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47, which are responsible for degradation of host DNA. Wiberg has studied phage mutants in gene 56 which are deficient in deoxycytidine triphosphatase and do not make DNA. He has shown that when these are coupled with phage mutants in genes 46 or 47, appreciable amounts of DNA are accumulated. Similarly it has been found that recombination of gene 30 with gene 46 results in accumulation of phage DNA and it is concluded that degradation of DNA in cells infected with gene 30 mutants occurs by the same mechanism responsible for the breakdown of host DNA. T4 mutants in gene 30, or in gene 30 recombined with gene 46 are capable of synthesis of late proteins, but mutants in gene 56, or in gene 56 recombined with gene 46 are not capable of this synthesis, (Hosoda and Levinthal, to be published).

To investigate the difference in stability of normal T4 DNA and the DNA made by amH39, several phage-specific enzyme activities which might make the phage DNA resistant to phage-induced DNAase(s) were assayed in an extract of cells of E. coli B infected with amH39. As shown in Table 1, this extract has no α-glucosyl transferase but has normal amounts of the β-glucosyl transferase. It is suggested that the absence of the α-glucosyl transferase in amH39 is not due to the mutation in gene 30 itself since another mutant in gene 30 (amE605) and the double amber mutant, obtained by crossing amH39 and amB3, induce normal amounts of both glucosyl transferases.

The α-glucosyl transferase defective mutant, T4αgt, was isolated from the original double mutant amH39 by the following procedure. E. coli cells were mixedly infected with equal amounts of wild-type T4 (++) and amH39 (amH39X, gt).* The progeny phages were plated on E. coli CR63, the permissive host for the amber mutant. Phages derived from some of the resultant plaques were tested for the presence of the amber mutation by plating on E. coli B and on CR63, and for their ability to make α-glucosyl transferase after infection of E. coli B.

*The symbol amH39X designates the new phage strain carrying the amber mutation in gene 30 which has been freed of the α-glucosyl transferase defect by crossing with wild type phage.

TABLE I

Phage	Defective Genes	Time after Infection (minutes)	α-glucosyl Transferase Activity	β-glucosyl Transferase Activity
T4 wild-type		5	20.9	58.3
		10	69.7	86.6
		15	100.0	100.0
		30	100.0	81.1
amH39	30, gt	5	0	43.1
		10	0	88.3
		15	0	109.7
		30	0	89.0
VX 3*	30, 46	30	98.2	143.7
amE605	30	30	142	102.3
amB3	46	30	89	85.8

E. coli B cells at 4×10^8 in tryptone-yeast extract broth were infected with 5 phages per bacterium and incubated at 37°. At the time indicated in the table, 2 ml of aliquots were pipetted out to the cold tube containing 20 μmoles NaN_3 and 1.8 mg of bovine serum albumin. Cells were sonicated for 1 minute with an MSE ultrasonic disintegrater. Both α- and β-glucosyl transferase activities were assayed as described by Josse and Kornberg, (1962) and all enzyme activities have been normalized to the activity induced by wild type phage at 15 minutes taken as 100 per cent. C^{14} UDPG and DNA of T2 gt as substrates were kindly supplied by Dr. H. Revel. The phage strain amH39, amE605 and amB3 were obtained from Dr. R. Edgar. *VX3 was made by crossing amH39 and amB3 in this laboratory.

TABLE II

Bacteria	Phage	α-glucosyl Transferase Activity
E. coli B.	T4 wild type	100.0
	H39X	100.6
	gt	0
CR63	H39X	83.2
	gt	0.5

The conditions of the experiment were the same as in Table I. Extracts were made from cultures 20 minutes after infection. Activity in E. coli B infected with wild-type T4D is set at 100.

The two mutations segregated to give the following results: among 18 plaques tested: 6 + +, 6 + gt, 3 amH39X + and 3 amH39X gt. The α-glucosyl transferase mutation is not of the amber type since T4αgt does not induce the enzyme in either E. coli CR63 or B (Table II). T4αgt DNA, glucosylated only by the β-glucosyl transferase, is stable. The instability of the DNA made by the original amH39 appears to be due to the amber mutation in gene 30 since DNA synthesized by the recombinant amH39X is degraded in a similar fashion.

T4αgt isolated from amH39 differs from the α-glucosyl transferase mutants of T2 and T6 (T2gt and T6gt) recently isolated by Revel et al., (1965) in that it has the same efficiency of plating on E. coli B as on Shigella dysenteriae Sh. It has been shown by Hattman and Fukasawa (1963) that T-even phages which lack glucose on their DNA (T^* phages) are characterized by their ability to grow in Sh (permissive host) but not in B (restrictive host). In T2 and T6 extensive glucosylation of phage DNA is dependent upon a single enzyme, α-glucosyl transferase. Thus loss of this enzyme gives rise to phage containing non-glucosylated DNA which are restricted in E. coli B. In contrast glucosylation of T4 DNA is accomplished by 2 enzymes: an α-glucosyl transferase which adds glucose in α-configuration to 70% of the HMC residues and a β-glucosyl transferase which adds glucose in β-configuration to the remaining 30% of the HMC residues (Josse and Kornberg, 1962; Lehman and Pratt, 1960). It is not known to what extent the DNA of T4αgt is glucosylated by the β-glucosyl transferase. Studies of the α and β enzymes of T4 in vitro have shown that glucosylation of HMC-DNA by the β enzyme is more extensive than by the α enzyme: the β enzyme can glucosylate all available HMC residues (Kornberg et al., 1961). The specificity of the 2 transferases is not governed by the sequence of bases of the substrate DNA in vitro (Josse and Kornberg, 1962). The mechanism by which β-glucosylation of normal T4 DNA is fixed at 30% is not known.

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