SELECTED GENES OF T_7 DNA ASSOCIATED WITH THE *ESCHERICHIA COLI* MEMBRANE FOLLOWING PHAGE INFECTION

Dag HELLAND and Agnar P. NYGAARD
Department of Biochemistry, University of Bergen, Bergen, Norway

Received 11 November 1974

1. Introduction

At present there are no rigorous criteria to define a membrane–DNA complex. The techniques used for their recovery involve either rapid sedimentation or association with Mg-sarcosyl crystals. Using these methods the parental DNA of those phages studied (including T_4, λ (in the absence of repressor), φ X 174, and T_7) appears to be attached to the cell membrane during vegetative reproduction. Furthermore, synthesis of viral progeny DNA seems to take place on the membrane in several cases (for review see [1]). It has been shown that specific genes are involved in the binding of T_4 DNA [2] as well as λ DNA [3] to the membrane.

In this laboratory a rapidly sedimenting complex isolated from T_4 and T_7 infected *E. coli* has been studied. This complex contains a large portion of the total RNA polymerase activity (EC 2.7.7.6), and the associated DNA serves as an efficient template for the enzyme [4]. In the 'early' period of T_4 infection the membrane appears to be enriched in 'early' genes [5]. The present report contains a corresponding study of early and late genes in T_7 membrane bound DNA following infection.

2. Materials and methods

All chemicals used were of highest commercial grade. [5-^{3}H]-Uracil (29 Ci/mmole) and [5Me-^{3}H]-thymidine (15.7 Ci/mmole) were obtained from the Radiochemical Centre England, Unisolve-l from Koch Light Lab., England, pronase B from Calbiochem, USA, pancreatic RNA-ase and pancreatic DNA-ase were purchased from Sigma Chemical Co. USA.

A preparation of *E. coli* core RNA polymerase (EC 2.7.7.6) was a gift from Dr A. K. Abraham.

E. coli B was grown at 30°C in casamino acids glycerol medium [6] to a concentration of 5×10^8/ml and was then infected with phage T_7 using a multiplicity of infection of 20.

In order to inhibit protein synthesis in phage infected cells chloramphenicol was added to a concentration of 400 μg/ml 5 min before infection. The cells were harvested 7 min after infection. (CAM) DNA and (CAM) RNA were prepared from these cells.

E. coli RNA was labelled by the addition of ^{3}H-uracil (1 mCi/ml) for 4 min during exponential growth of the cells. The preparation contained 2100 cpm/μg.

Phage RNA was labelled between 14 and 17 min after infection by the addition of 1 mCi/ml of ^{3}H-uracil to the medium. The preparation contained 1750 cpm/μg.

RNA was isolated from lysozyme and sodium dodecyl sulfate-treated cells using hot phenol extraction.

RNA was synthesized using core RNA polymerase (50 μg/ml) [7] and with either denatured T_7 phage particle DNA or denatured 7' membrane DNA as templates. The conditions were as follows: 0.4 mM ATP, 0.2 mM GTP, 0.2 mM CTP, ^{3}H-labelled 0.05 mM UTP containing 0.05 mCi; 30 mM Tris–HCl, 2.5 mM orthophosphate, 150 mM KCl, 10 mM MgCl, 0.1 mg/ml bovine serum albumin, 10 μg/ml DNA. The solutions were incubated for 30 at 37°C.

RNA was extracted with hot phenol, DNA was degraded by incubation with 25 μg/ml DNA-ase I (E. P.) for 30 at 37°C. DNA-ase was subsequently removed by phenol extraction.

Approximately 50% of the labelled material formed hybrid with excess denatured T_7 DNA. Phage was pu-

rified according to Ymamoto and Alberts [8]. The membrane fraction was prepared from osmotically-lysed spheroplasts according to Haarr [9]. DNA was isolated from T_7 phage, from the membrane preparation, and from whole cells according to methods described in references [10], [11] and [12] respectively. Protein concentration was estimated by the Lowry method [13].

Solutions of denatured DNA and labelled RNA were incubated for 4 hr at 67°C in 0.01 M Tris–HCl, pH 7.3, and 0.5 M KCl. Hybrids were collected on nitrocellulose membrane filters and counted [14].

3. Results

3.1. *E. coli DNA and T_7 DNA of the membrane preparation*

Figs. 1 and 2 show approximate values for total DNA and *E. coli* DNA, respectively, isolated from the membrane fraction following T_7 infection.

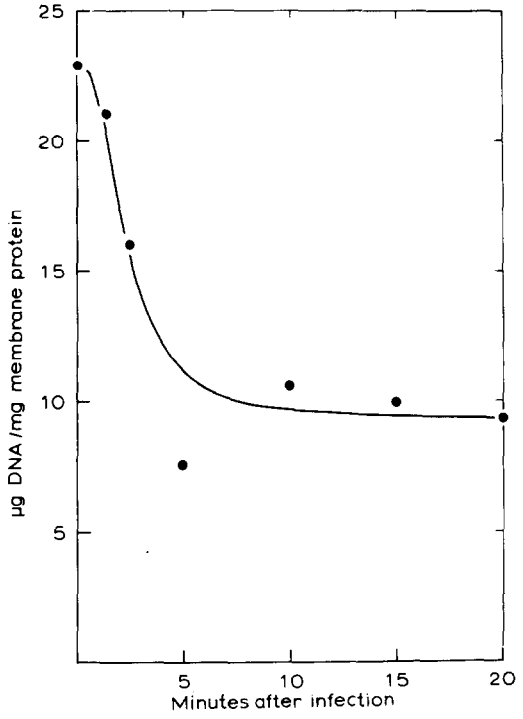

Fig.1. Total DNA in the membrane following infection. Membrane preparations isolated at varying times after infection were incubated in 0.3 N KOH for 10 hr, neutralized, and two vol of ethanol were added at 0°C. The precipitate was collected, extracted with 5% trichloroacetic acid at 90°C for 20′, and DNA was determined in the supernatant according to Burton [15] using calf thymus DNA as standard.

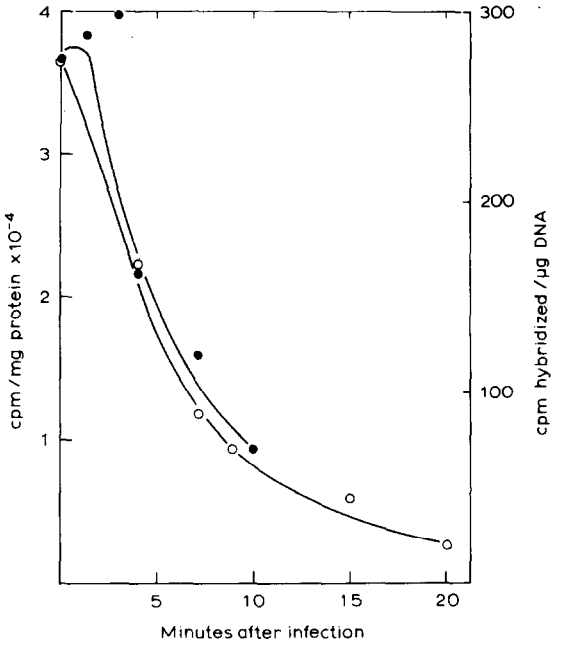

Fig.2. *E. coli* DNA in the membrane following infection. (o—o—o) Decrease of universally labelled *E. coli* DNA/mg membrane protein (ordinate, left) *E. coli* cells were grown in the presence of 250 μg/ml deoxyadenosine and 1 μg/ml 5Me-[^{3}H]-thymidine [16]. Labelling was interrupted at the time of infection. The isolated membrane preparations were dissolved in 20 vols Cellosolve-1 and the radioactivity was determined directly (see Materials and methods). (•—•—•) Capacity to hybridize with excess *E. coli* RNA/μg total membrane DNA (ordinate, right). Approximately 2 μg/ml of membrane DNA isolated at differing times after the infection was hybridized with ^{3}H-labelled *E. coli* RNA. Maximal hybridization was obtained with approximately 100 μg RNA/ml (see Materials and methods).

Approximately 20% of the [5Me-^{3}H]-thymidine incorporated into phage particle DNA was found to be associated with the membrane at 3, 6, 10, 13 and 18 min after infection. Furthermore, when [5Me-^{3}H]-thymidine was added to the cells 6′ after infection approximately 20% of the label appeared in the membrane at 9, 12 and 17 min after infection (conditions as described in legend to fig.2a). Thus it appears that approximately one fifth of parental DNA and one fifth of replicated DNA [17] is associated with the membrane.

3.2. *Characterization of membrane bound T_7 DNA*

T_7 DNA was characterized by hybridization-competition experiments. It is known that T_7 infection gives rise to two distinct populations of phage RNA, 'early' and 'late' [17]. Only early RNA, copied from the left 20% of the genome is produced during the first 4–5 min, while both early and late are synthesized during the late period of infection (e.g. 14–17 min) (for review see [18]). Thus, when T_7 DNA containing both early and late genes, is hybridized with labelled 14–17 min RNA in the presence of increasing concentrations of unlabelled early RNA, maximally 20% of the hybrid should be competed out. In contrast, with a genome containing only (or mainly) early T_7 genes, the competition should be complete (or nearly so). Fig.3 shows the results obtained with differing membrane DNAs, with phage particle DNA, and with total DNA isolated from infected cells. Clearly, membrane bound T_7 DNA contains mainly early genes except when isolated very early and very late in the infection period (1.5, 19 and 20 min), and when isolated from cells inhibited by chloramphenicol.

In order to confirm the results indicating selection of genes, denatured DNA from the membrane, isolated 12 min after infection, and denatured T_7 phage particle DNA were used as templates for the synthesis of RNA by core RNA polymerase, and the products examined by competition experiments. While DNA from the membrane would be expected to produce only early RNA, all types of RNA should be synthesized using denatured T_7 phage particle DNA as a template under these conditions [8]. The isolated H-strand of T_7 DNA [19] was used for hybridization, since this is the only strand which hybridizes with in vivo RNA (20). The results are shown in fig.4. Nearly complete competition is observed between RNA synthesized in vitro with membrane DNA as a template and early in vivo RNA, whereas only partial competition is obtained between RNA produced with T_7 phage particle DNA and early in vivo RNA. Thus, denatured

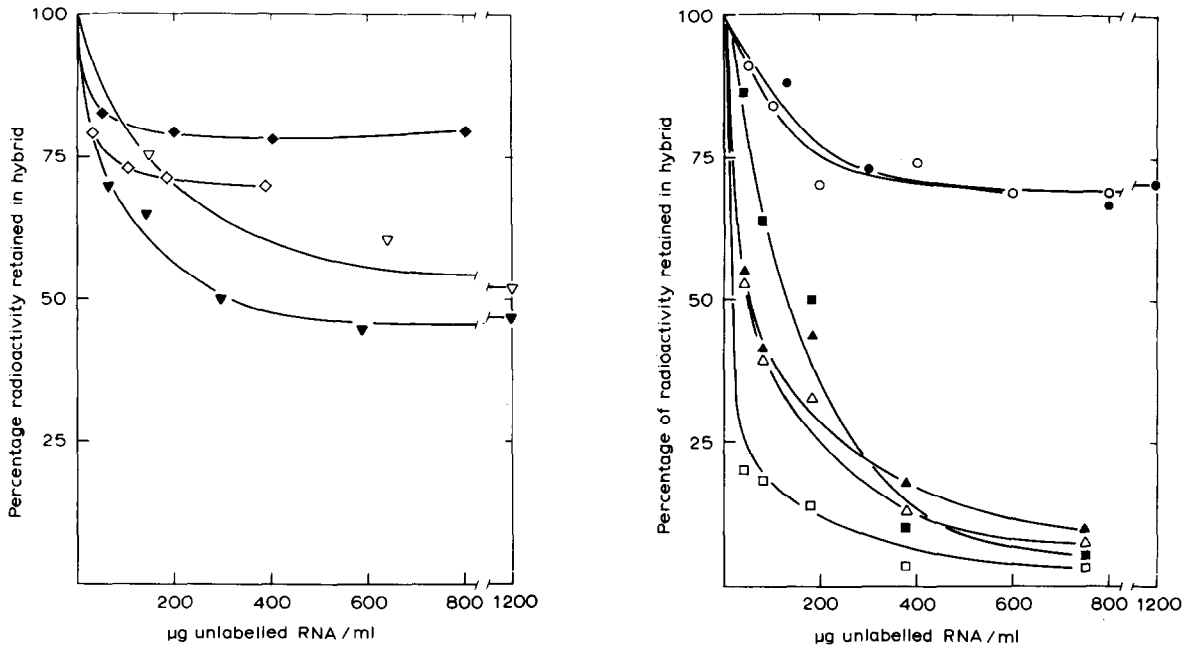

Fig.3a and b. Characterization of DNA by hybridization-competition. The solutions contained: denatured DNA; 14–17 min labelled T_7 RNA, 64 µg/ml; unlabelled (CAM) RNA in varying concentrations. 100% corresponds to 600–1000 cpm/250 µl. For further details see Materials and methods.

a) ▼5.1 µg/ml membrane DNA, 1.5 min; ◇3.1 µg/ml membrane DNA, 19 min; ◆2.5 µg/ml membrane DNA, 20 min; ▽16.7 µg/ml (CAM) DNA from membrane; b) ▲3.0 µg/ml membrane DNA 2.5 min; △2.9 µg/ml membrane DNA, 7 min; ■1.6 µg/ml membrane DNA, 12 min; □1.9 µg/ml membrane DNA, 15 min; ○2.8 µg/ml phage particle DNA; ●38 µg/ml DNA from 15 min whole cells.

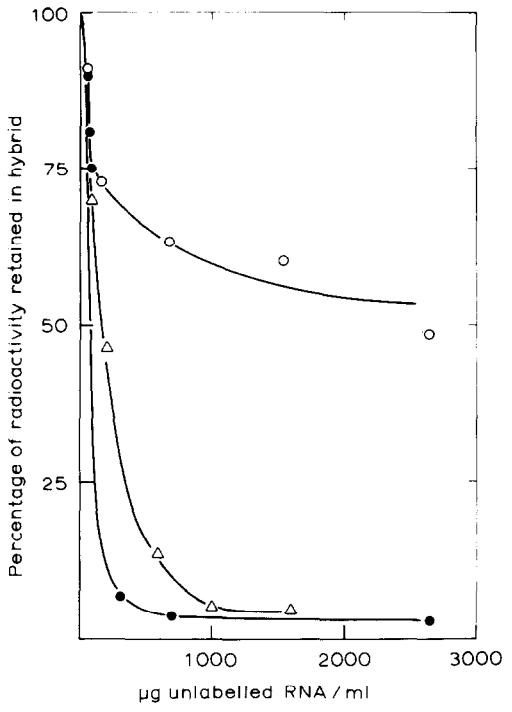

Fig.4. Characterization of in vitro RNA by hybridization-competition. All the solutions contained: 1 μg/ml of the H-strand of T_7 phage particle DNA [19]. 100% corresponds to approx. 300 cpm/250 μl. For further details see Materials and methods. Labelled RNA synthesized using denatured T_7 phage particle as a template. (o—o—o) Competed with unlabelled (CAM) RNA. (△—△—△) Competed with unlabelled 17 min T_7 RNA. (●—●—●) Labelled RNA synthesized using denatured 7 min membrane DNA as a template. Competed with unlabelled (CAM) RNA.

DNA from the membrane serves as template for only early RNA in the core RNA polymerase system and is therefore likely to contain early genes only.

4. Discussion and results

The fact that only early genes are present in our membrane preparations between 2.5 and 15 min after infection could be due to detachment of late genes by shearing, or the action of nucleases during the preparation procedure. This would imply that early genes are specifically protected by binding to the membrane. Very early in the infection period (1′) and in chloramphenicol treated cells, nucleases may be less active. In the very late period, phage proteins, which are believed to be incorporated into the membrane [1], could improve the protection of the whole genome.

The results strongly indicate that early T_7 genes are specifically bound to the membrane during the infection period.

References

[1] Siegel, P. J. and Schaechter, M. (1973) Ann. Rev. Microbiol. 27, 261–282.
[2] Marsh, R. C., Breschkin, A. M. and Mosig, G. (1971) J. Mol. Biol. 60, 213–233.
[3] Tramblay, G. Y., Daniels, M. J. and Schaechter, M. (1969) J. Mol. Biol. 40, 65–76.
[4] Haarr, L. and Nygaard, A. P. (1973) Abstr. 9 Int. Congress of Biochem. p. 165.
[5] Jörstad, K. E., Haarr, L. and Nygaard, A. P. (1973) Abstr. 9 Int. Congress of Biochem. p. 194.
[6] Frazer, D. and Jerrel, L. A. (1953) J. Biol. Chem. 205, 291–295.
[7] Bautz, E. K. F., Bautz, F. A. and Dunn, J. J. (1969) Nature 223, 1022–1024.
[8] Yamamoto, K. R., Alberts, B. M., Benziger, R., Lawhorne, L. and Trieber, G. (1970) Virology 40, 734–744.
[9] Haarr, L. (1967) Biochim. Biophys. Acta 145, 202–204.
[10] Grossman, L., Levine, S. S. and Allison, W. S. (1961) J. Mol. Biol. 3, 47–60.
[11] Massie, H. R. and Zimm, B. H. (1965) Proc. Natl. Acad. Sci. U. S., 54, 1636–1643.
[12] Kelly, T. J., Jr. and Thomas, C. A., Jr. (1969) J. Mol. Biol. 44, 459–475.
[13] Lowry, W. C., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[14] Nygaard, A. P. and Hall, B. D. (1964) J. Mol. Biol. 9, 125–142.
[15] Burton, K. (1956) Biochem. J. 62, 315–323.
[16] Boyce, R. P. and Setlow, R. B. (1962) Biochim. Biophys. Acta 61, 618–620.
[17] Siegel, R. B. and Summers, W. C. (1970) J. Mol. Biol. 49, 115–123.
[18] Studier, F. W. (1972) Science 176, 367–376.
[19] Szybalski, W., Kubinski, H., Hradecna, Z. and Summers, W. C. (1971) Methods in Enzymology 21, part D, 383–413.
[20] Summers, W. C. and Szybalski, W. (1968) Virology 34, 9–16.