CELL FREE SYNTHESIS OF BACTERIOPHAGE LAMBDA REPLICATION PROTEINS

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1. Introduction

Bacteriophage lambda codes for two functions directly involved in the replication for its DNA: the products of genes *O* and *P* [1]. These two genes are transcribed to give a single polycistronic messenger RNA [2]. A third essential element, the origin of replication, is thought to be a structure on the DNA in the region where replication of the bacteriophage chromosome starts [3]. In conjunction with host factors, the replication proteins are believed to activate the origin and lead to initiation of the DNA replication.

Using an in vitro complementation system it has been possible to obtain protein fractions displaying *P*-activity [4]. With the *O*-gene product the same has not been achieved.

The *O*-activity is known to have a short half-life in vivo [5], whereas the *P*-activity is more stable.

This fact can be explained in terms of a requirement of different amounts of the two proteins in the cell, different rates of their synthesis (i.e., translational control), an inherent instability of the *O*-protein, or a combination of these factors.

We have set out to synthesize the two λ replication proteins in vitro using a DNA-dependent cell free protein synthesizing system [6]. Employing DNA from wild type and amber mutant λ phage we will show that under these conditions the *O*-protein is synthesized in sizeable amounts while the *P*-protein is hardly detectable. This suggests an instability of the *O*-protein as a reason for the rapid decay of its activity in the cell and translational control of the synthesis of the two replication proteins.

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2. Materials and methods

Cell-free protein synthesis was performed as described before [6] using spermidine at a concentration of 3 mM, $MgCl_2$ at 8 mM, and phosphoenolpyruvate at 10 mM. The DNA of phages λ CIb2 (called λ wild type, λ wt below) and the otherwise isogenic amber mutants 08, 0125, P3 and P80 [7] was prepared according to Miller [8]. λ dv-DNA was isolated as covalently closed circular DNA [9]. The protein synthesis reaction was stopped after 45 min at 35°C by application to Sephadex G-25 columns and desalted [10]. Electrophoretic analysis of the products was performed on 12.5% polyacrylamide–SDS-gels [11] with subsequent fluorography [12].

3. Results and discussion

The transcription of the λ genome is controlled in a very complex fashion. Two major positive control elements, the products of genes *N* and *Q*, govern the expression of early and late genes, respectively [13].

To investigate the influence of these two gene products on the in vitro transcription–translation system we have used equimolar amounts of λ wt or λ dv DNA as templates. λ dv is a plasmid which lacks the *N*-gene, early genes to the left of *N*, and the late genes [14].

Figure 1 shows that the λ specific products obtained in the protein synthesis assay are qualitatively the same in both cases. This means that transcription and translation of early genes is obtained in the absence of the *N*-gene product. It also implies that late genes are not produced, possibly because the *N*-product is not synthesized in sufficient amounts in vitro,

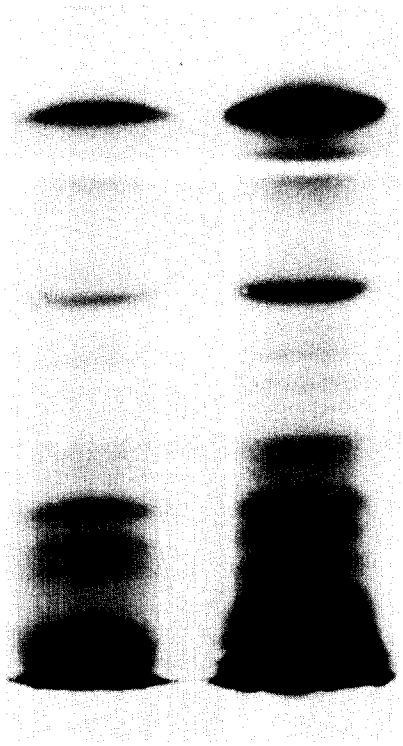

Fig.1. Electrophoretic analysis of proteins synthesized in vitro using wt DNA or dv DNA templates. Equimolar amounts of DNA were used (3 pmol/ml).

even when the *N*-gene is intact. The higher amount of protein obtained when λ dv DNA was used as template might be explained on the basis of either the supercoiled state of this circular DNA, which is known to lead to promoter activation [15], or its resistance to nucleases.

The relatively simple pattern of λ-specific protein observed in the assay employing whole λ wt DNA made it possible to directly compare the proteins synthesized in systems containing either wild type or amber-mutant DNA. The results of such an experiment are seen in fig.2. It is apparent that the DNAs of amber mutants 0125 and 08 lead to the production of fragments of a slow migrating major protein formed on wild type and *P*-mutant DNAs. This protein can thus be identified as the *O*-protein. It is seen as a fairly strong band in the wild type and *P*-mutant cases at a molecular weight, graphically determined [16], to be approx. 37 000 (fig.3). This value agrees reasonably well with that of Yates et al. [17] who have independently estimated the molecular weight of the *O*-protein to be 34 500. The sizes of 35 000 and 26 000 calculated from our data for the 0125- and 08-fragments, respectively, are consistent with the order of the two mutational sites in the *O*-gene, 0125 being operator distal relative to 08 [7].

At a position corresponding to a mol. wt approx. 27 000, a band can be seen both with the wild type and 0125 DNA (figs 1 and 2). When λ 08 DNA is used, it is covered up by the *O*-amber fragment. In the case of the *P*-amber mutants only very faint bands are visible in a similar position. We thus estimate the molecular weight of the *P*-protein to be 27 000. The lack of visible *P*-amber fragments can be explained in two ways: the fragments of the *P*-protein mutants may be too short to be resolved from the small molecular material found on the lower edge of the gel [18] or else they might be degraded in the system.

Assuming that our findings reflect the situation in vivo, we come to the following conclusions:

The *O*-protein is produced in amounts greater than other early λ proteins including the *P*-product. Since the *O*- and *P*-genes are transcribed into a polycistronic messenger-RNA the high amount of *O*-protein found relative to *P*-protein indicates that the protein is rapidly inactivated in the cell after its production. On the other hand, the low amount of *P*-product suggests either a translational control or a rapid decay of that part of the messenger-RNA which codes for the *P*-protein. This points to a low requirement of *P*-protein relative to *O*-protein in the cell.

An incomplete synthesis in vitro of the messenger-RNA coding for the *P*-function can most probably be ruled out as an explanation for the differential synthesis since using different DNA templates in the same system, proteins of molecular weights higher than 70 000 are produced in large quantity (data not shown), which requires efficient synthesis of long messenger-RNA molecules.

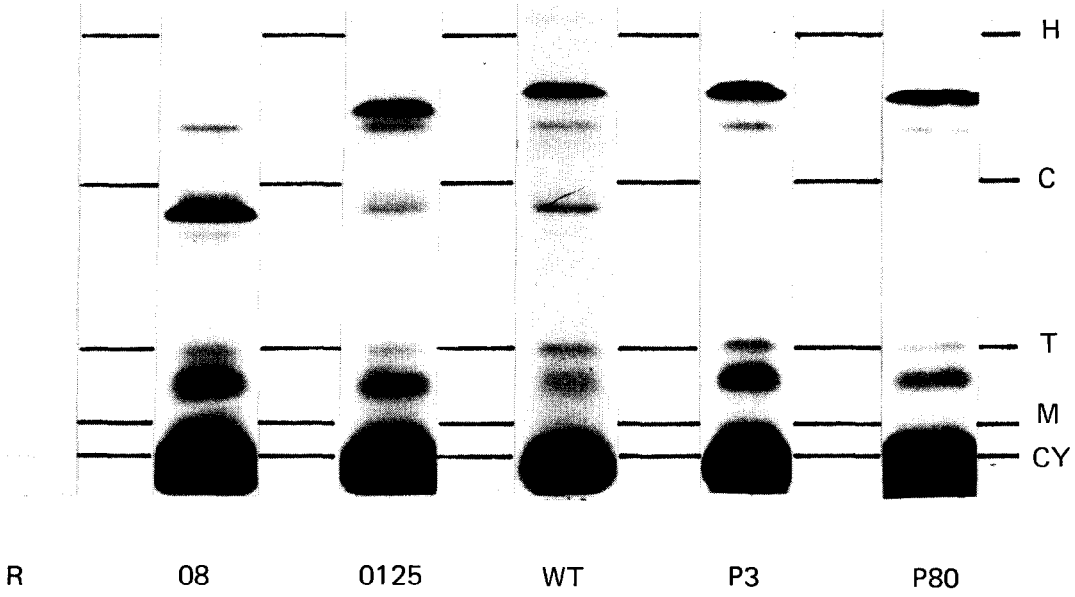

Fig.2. Electrophoretic analysis of proteins synthesized in vitro using wt and *O* or *P*-amber mutants DNA as templates. The horizontal lines indicate the positions of non-radioactive molecular weight standards: H, egg white albumin (mol. wt 45 000); C, carboanhydrase (mol. wt 29 000); T, trypsin inhibitor (mol. wt 21 000); M, myoglobin (mol. wt 17 800); Cy, cytochrome *c* (mol. wt 12 400). R is a pattern of a control reaction run without added DNA.

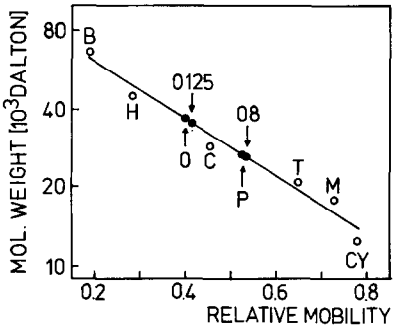

Fig.3. Determination of the molecular weights of proteins synthesized in vitro (see fig.2). The marker proteins are those used in fig.2 and bovine serum albumin (B, mol. wt 67 000).

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References

[1] Brooks, K. (1965) Virology 26, 489–499.
[2] Szybalski, W., Bøvre, K., Fiandt, M., Hayes, S., Hradecna, Z., Kumar, S., Lozeron, H. A., Nijkamp, H. J. J. and Stevens, W. (1970) Cold Spring Harbor Symp. Quant. Biol. 35, 341–353.
[3] Stevens, W. F., Adhya, S. and Szybalski, W. (1971) in: The Bacteriophage Lambda (Hershey, A. D. ed) pp. 515–533, Cold Spring Harbor Laboratory.
[4] Klein, A., Bremer, B., Kluding, H. and Symmons, P. (1977) submitted.
[5] Wyatt, W. M. and Inokuchi, H. (1974) Virology 58, 313–315.
[6] Fuchs, E. (1976) Eur. J. Biochem. 63, 15–22.
[7] Campbell, A. (1971) in: The Bacteriophage Lambda (Hershey, A. D. ed) pp. 13–44, Cold Spring Harbor Laboratory.
[8] Miller, J. H. (1972) in: Experiments in Molecular Genetics, pp. 333–334, Cold Spring Harbor Laboratory.
[9] Hobom, G. and Hogness, D. S. (1974) J. Mol. Biol. 88, 65–87.
[10] Emneus, N. J. A. (1968) J. Chromat. 32, 243–257.
[11] Laemmli, U. K. (1970) Nature 227, 680–685.
[12] Laskey, R. A. and Mills, A. D. (1975) Eur. J. Biochem. 56, 335–341.

[13] Thomas, R. (1971) in: The Bacteriophage Lambda (Hershey, A. D. ed) pp. 211–219, Cold Spring Harbor Laboratory.
[14] Matsubara, K. and Kaiser, A. D. (1968) Cold Spring Harbor Symp. Quant. Biol. 33, 769–775.
[15] Botchan, P. (1976) J. Mol. Biol. 105, 161–176.
[16] Dunker, A. K. and Rueckert, R. R. (1969) J. Biol. Chem. 244, 5074–5080.
[17] Yates, J. L., Gette, W. R., Furth, M. E. and Nomura, M. (1977) Proc. Natl. Acad. Sci. USA 74, 689–693.
[18] Gesteland, R. F. and Kahn, C. (1972) Nature New Biol. 240, 3–6.