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SYNTHESIS AND DECAY OF λ DNA REPLICATION PROTEINS IN MINICELLS

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SUMMARY. The coliphage λ DNA replication proteins, the O- and P-gene products, have been identified by infection of nonpermissive Escherichia coli minicells with the appropriate λ amber mutants as proteins of a molecular weight of about 34000 and 23000, respectively. Proteins of exactly the same size were found in minicells harbouring the plasmid λdv. Both proteins seem to be synthesized at the same rate. In λ-infected minicells, as well as in λdv-harbouring minicells the pulse-and-chase experiments have shown an exceptionally rapid decay of the O-protein.

INTRODUCTION. It has been shown recently, that Escherichia coli minicells infected with bacteriophage λ synthesize λ-specific mRNA (1, 2) and proteins (3, 4), which can be easily detected since host macromolecular syntheses do not occur in this system. In this paper, we identify the O- and P-gene products, and study the in vivo synthesis and decay of these λ early proteins engaged in the initiation and maintenance of the λ DNA replication (5). Genetic experiments suggest that the O-protein interacts with both a specific target sequence in the DNA molecule (at or near the origin of replication), and the P-protein (6, 7, 8). There are also suggestions that the P-gene product interacts with several host proteins, the products of genes dnaA (9), dnaB, dnaJ, dnaK, grpD and grpE (10). Since O-protein fits a major criterion of an initiator, as defined in the replicon model of Jacob et al.(11), the identification of this protein in vivo and the study of its chemical stability seemed to us a matter of the utmost importance.

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MATERIALS AND METHODS. The bacterial strains were described previously (4). We have confirmed that the strains originating from P678-54 are *su2*$^+$ (12). The *su0* allele was introduced by interrupted mating with W3807 Hfr6 (13). The nonpermissive (*su0*) strain A265, producing mini[ColE1], was selected as a gal$^+$ recombinant. The phages λ*0am29*, λ*Pam3* (14) were from H.Echols, λ*v2v3vs326* (λ*svir*) from G.Ordal (15), λ*hy106* from S.Hilliker (16), and λ*cI72* from W.Szybalski. Phage-infection of minicells and protein labeling with [^{14}C]aminoacids were performed as described earlier (4). The labeling was terminated by layering over an equal volume of frozen 10 mM Tris-HCl/20 mM sodium azide buffer, pH 7.2, containing 0.05% NaCl. We have also used boiling in SDS for this purpose (17), but have not got better results. In the pulse-and-chase experiments the labeling was stopped by a 200-fold dilution of [^{14}C]aminoacids with the nonradioactive ones. The acrylamide (10%) - SDS slab gel electrophoresis of radioactive proteins was performed according to Laemmli (18). The fluorograms (19) were traced with a Vernon densitometer. The following served as standards (molecular weight in parentheses) : colicin E1 (56000), proteins of mature λ phage, pE (38000), pV (31000) and pD (12000), and bovine trypsin (23000).

RESULTS AND DISCUSSION. The initial approach to the identification of λ DNA replication proteins consisted in the application of the λ/P22 hybrid, λ*hy106*. In this phage the replicative region reveals the P22 specificity (16). One of the P22-specified proteins of a molecular weight of about 50000 can be easily recognized when λ*hy106*-infected minicells are examined (Fig.1). At least two proteins synthesized in λ-infected minicells were absent when λ*hy106* was used for infection (Fig.1); their molecular weights amounted to about 34000 and 23000. For the final identification the infection of nonpermissive minicells with λ amber mutants has been chosen. An infection with the *O*-gene mutant resulted in the disappearance of only one peak (Fig.2A,B), indicating a protein of a molecular weight of about 34000 as a product of the *O*-gene. An analogous examination of the *P*-gene amber mutant has revealed that the protein of a molecular weight of about 23000 is the *P*-gene product (Fig.2A,C). The P-protein has been already identified in the UV-irradiated bacterial cells (20).

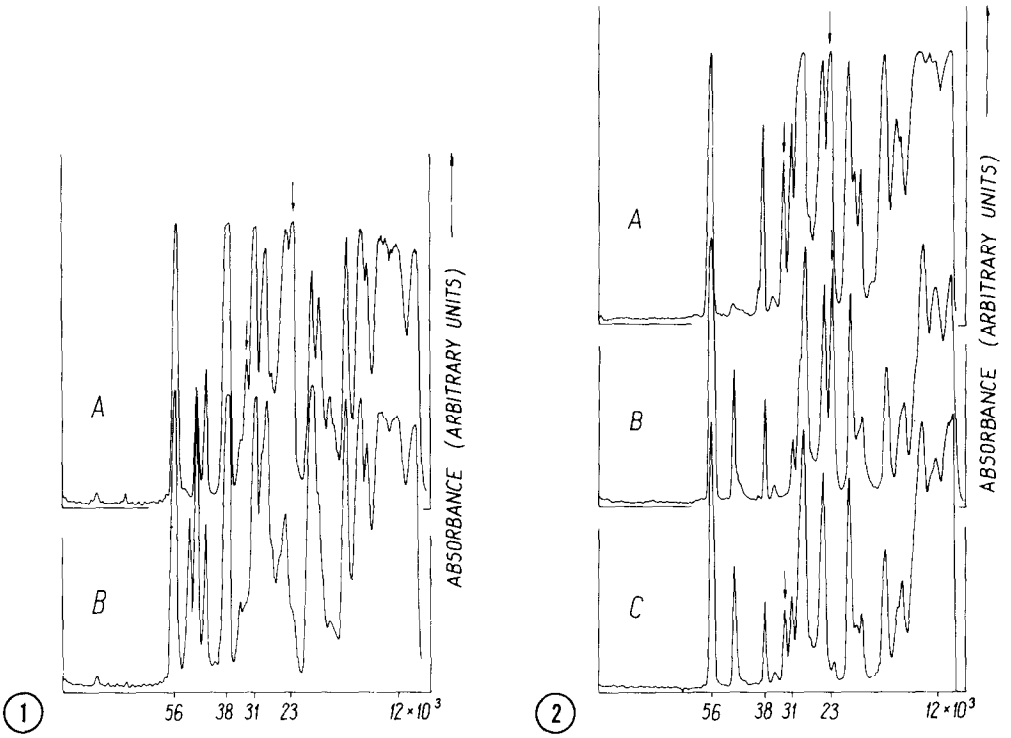

Fig.1. Tentative identification of λ replicative gene-products. Acrylamide gel electrophoresis of proteins synthesized in mini[ColE1]su2⁺ infected with λcI72 (A), or λhy106 (B). The arrows point to the proteins which are not synthesized during λhy106 infection. Since this hybrid phage lacks λ replicative region, these proteins are likely products of the λ genes O and P. The minicells were labeled with [^{14}C]aminoacids for 10 minutes after infection.

Fig.2. Final identification of the O- and P-gene products. Acrylamide gel electrophoresis of proteins synthesized in mini[ColE1]su0 infected with λcI72 (A), λOam29 (B) and λcIts857Pam3 (C). The arrows point to the O- and P-gene products (molecular weights of 34000 and 23000, respectively) which are not synthesized when the appropriate λ amber mutants are used for infection. The post-infection period of labeling with [^{14}C] aminoacids amounted to 10 minutes.

The synthesis of O- and P-gene products in minicells, observed early after the addition of the label (2 min pulse, Fig.3), as well as the finding that proteins of similar size are specified by these genes in an *in vitro* experiment (21, 22) proves that proteins of molecular weights of 34000 and 23000 are the direct products of translation. Both λ replication genes are transcribed to give a single polycistronic mRNA (23), hence any

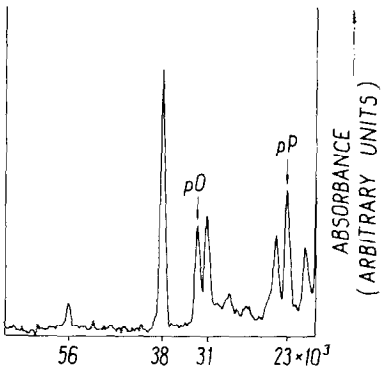

Fig.3. Short pulse labeling of λ-infected minicells. Acrylamide gel electrophoresis of proteins synthesized in mini[ColE1] infected with λsvir. The proteins were labeled with [^{14}C]aminoacids between the 10th and 12th min after infection. The O/P ratio is considerably higher than that in the preceding figures, when a longer labeling period was applied.

difference in the rate of synthesis of these proteins may be interpreted as the manifestation of a posttranscriptional control. Short pulse experiments revealed that the net synthesis of the O-protein occurs nearly at the same rate as in the case of the P-protein (Fig.3). Taking into account the extreme instability of the O-protein (see next paragraph) which must lower the O-protein peak even during a short pulse, we conclude that both λ replication proteins are synthesized at the same rate in λ-infected minicells. In this context it is worth to recall that in the DNA-dependent coupled protein synthesis, the O-protein was synthesized in considerable amounts while the P-protein was hardly detectable (22); it seems, therefore, that this finding did not reflect the situation in vivo.

The most interesting question concerning the reason for the instability of the O-gene activity (24) has been answered by the pulse-and-chase experiments. The O-protein faded out very fast (Fig.4). The chemical half-life (uncorrected) of this protein at 42°C amounted to 4 minutes (Fig.5). However, in the case of

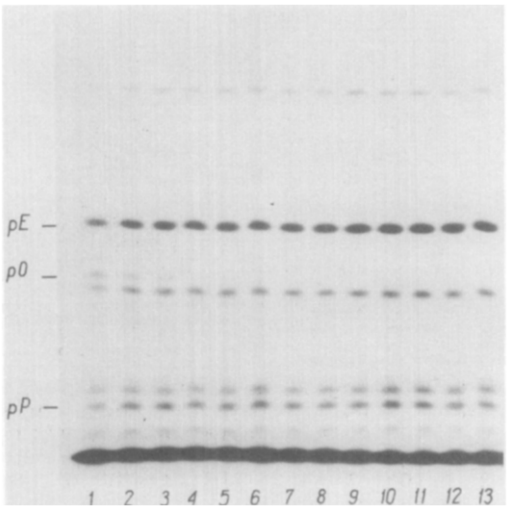

Fig.4. The pulse-and-chase experiment. The λ-infected minicells, labeled as indicated in Fig.3 (lane 1), were incubated with nonradioactive aminoacids for : 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 min (lanes 2 - 13). Note the rapid disappearance of the O-protein.

other λ-coded proteins, supposed to be stable, we observed a residual isotope-incorporation after the end of the pulse, issuing probably from the intracellular pool of isotope-labeled aminoac-

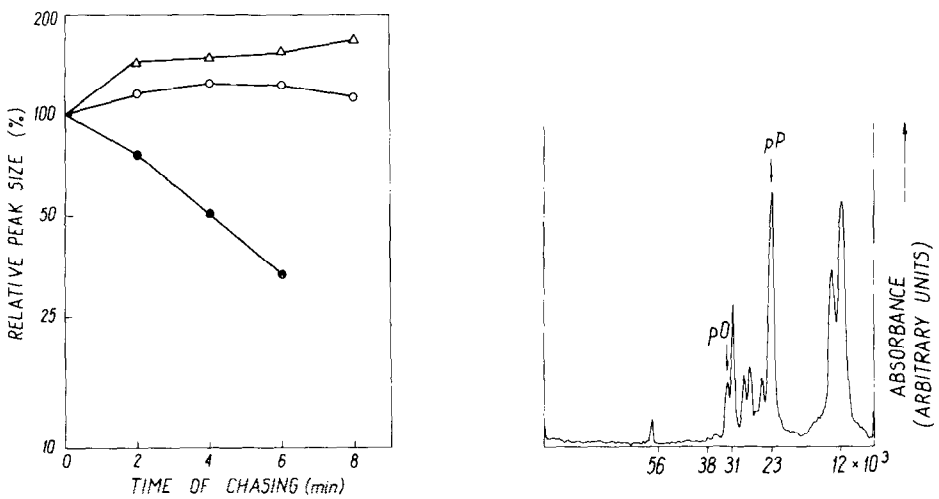

Fig.5. Kinetics of decay of λ DNA replication proteins, pO (•) and pP (o), and of λ major head protein pE ( ). The relative peak size was determined by scanning the fluorogram presented in Fig.4 and by integrating the areas under the peaks.

Fig.6. The O- and P-gene products coded by the plasmid λdv1. The minicells harboring the plasmid λdv1 were labeled with [^{14}C] aminoacids for 30 min. The arrows point to the λ DNA replication proteins, pO and pP.

ids. This incorporation ought to be taken into account in the case of the O-gene product, in order to obtain a real picture of the decay of this protein. The corrected value for the chemical half-life of O-protein at 42°C is about 2 min when P-protein serves as a reference, and even smaller when the residual E-protein synthesis is considered in calculations. Analogous experiments performed for longer time periods have revealed that the P-protein is stable; its chemical half-life exceeds 60 minutes.

The O- and P-gene products ought to be synthesized in minicells harbouring λdv plasmids, since these small replicons use the same proteins for their replication. This turned to be true: proteins of exactly the same size are specified by the λdv1 plasmid (Fig.6). Also in this case the O-protein appeared to be very labile (results not shown).

The rapid degradation of the initiator protein may be of fundamental importance in the control of the initiation of DNA replication.

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