VIRAL PROTEIN SYNTHESIS DURING REPLICATION OF BACTERIOPHAGE T1

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SUMMARY

During development of bacteriophage T1 at least 28 viral proteins are synthesized. Depending on the time of appearance, these polypeptides have been divided into three classes termed I, II and III. Second and third class proteins include the 13 structural monomers that make up T1 virion. A few of these proteins are synthesized before the onset of DNA replication. The molecular weights of the structural polypeptides range from 79,000 to 14,000 daltons. Pulse-chase experiments have shown that the major structural protein is synthesized in the form of a larger precursor which is then cleaved to give a final product of 30,000 daltons.

INTRODUCTION

For unknown reasons among the collection of bacterial viruses that belong to the T-series only T1 has been selectively neglected. As a matter of fact, not only the molecular mechanisms of its replication are completely obscure, but even the size of T1 virion is still to be assessed with some degree of confidence (1). Recently the existing genetic data concerning this phage (2,3) have been functionally extended (4,5) so that a preliminary comprehension of the physiology of T1 development is beginning to emerge. The present study was undertaken to define the structural polypeptides of the purified T1 virion and to analyse the overall pattern of T1-coded protein synthesis.

MATERIALS AND METHODS

Wild-type T1 bacteriophage and its host *Escherichia coli* B were obtained from Dr.M.Stodolsky. M9 medium (6) containing 0.5% glucose was used throughout. The concentration of sulfate in M9 was reduced to 0.05 M when the added label was [$Na_2{}^{35}SO_4$]. For radioactive phage production and purification *E.coli* B was grown at 37°C in M9. When the cell concentration was 5×10^8 per ml the bacteria were harvested, resuspended at the same density in fresh medium and infected with T1 at a multiplicity of 10. After 5 min carrier free [$^{35}SO_4$] or [^{14}C] - amino acid mixture was added to 10 μCi or 0.05 μCi per ml respectively and the culture was shaken until lysis occurred. The phage was purified by two cycles of differential centrifuga-

tion at low (5000 rpm for 10 min) and high speed (35,000 rpm per 90 min) in the 30 rotor of a Spinco L2-50 followed by isopycnic banding in a CsCl density gradient performed as described previously (7). Protein synthesis in infected cells was followed by adding [$^{35}SO_4$] (20 µCi per ml) or [^{14}C] aminoacids (2 µCi per ml) to T1-infected cultures for the prescribed time and terminating the pulse by chilling with ice containing 0.2 M NaN_3 either immediately or after a period of chase with nonradioactive precursor (2% Casaminoacids and or 0.1 M $MgSO_4$, final concentration). In most experiments to suppress host protein synthesis, the bacteria were irradiated with a 30 Watt UV lamp located 40 cm above for 5 min (8). The cells were lysed according to the method of Laemmli (9) and the radioactive protein samples were subjected to electrophoresis in SDS-polyacrylamide slab gels under the conditions specified by Blattler and Coll. (10). Autoradiography was performed as detailed by Fairbanks and Coll. (11).

RESULTS

Structural proteins of T1. Purified radioactive T1 bacteriophage after disruption and electrophoresis on 20% SDS-polyacrylamide gels gave rise to a reproducible autoradiographic pattern in which at least 13 structural polypeptides were resolved (Fig.1). Four bands, V8,V10,V12 and V13 contained about 75% of the total radioactivity. The molecular weight distribution of these proteins was estimated by coelectrophoresis with nonradioactive markers (12-14) and the approximate figures determined in this way are shown in Table I. The sum of these molecular weights is 540,000 daltons. Since the phage DNA has enough information to code for 1.8×10^6 daltons of protein (15) about 30% of T1 genome is needed to specify structural products.

Protein synthesis during viral infection. The overall pattern of T1 gene expression could be inferred from analysis of autoradio-

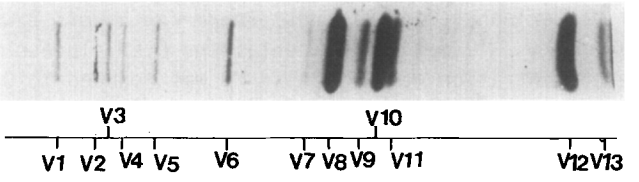

Fig. 1 Autoradiogram of a 20% polyacrylamide gel containing T1 structural proteins labelled with [^{35}S] . Migration was from left to right. Protein subunits are named V1 to V13.

grams of proteins synthesized during infection of unirradiated *E.coli* B (not shown). However, the presence of a remarkable host background rendered these experiments unsuitable for a neat definition of T1 protein synthesis regulation. UV irradiation of *E.coli* B was therefore used to reduce the host background levels (8). Under these conditions T1 DNA synthesis started 5-6 min after infection and lysis began 19 min later. The protein pattern from a sequence of 2-min pulses with $[^{35}SO_4]$ is shown in Fig.2 and summarized in schematic form in Fig.3. While only low molecular weight polypeptides were produced by UV-treated uninfected cells, after infection with T1 at least 28 phage-directed proteins were detected in gels. The essential feature of the pattern whose the sequen-

TABLE I

ESTIMATED MOLECULAR WEIGHTS OF T1 PROTEINS

Band	Molecular weight[a]	% of label in band[b]
V 1	79,000	1.1
V 2	70,000	2.2
V 3	68,000	0.5
V 4	52,000	1.7
V 5	49,000	2.4
V 6	44,000	7.4
V 7	41,000	0.6
V 8	30,000	25.6
V 9	27,000	5.8
V 10	26,000	15.5
V 11	24,000	3.3
V 12	16,000	23.2
V 13	14,000	10.7

a: Average of three separate runs performed as specified in Fig. 1

b: Relative proportions were obtained from tracings of autoradiograms by cutting and weighing.

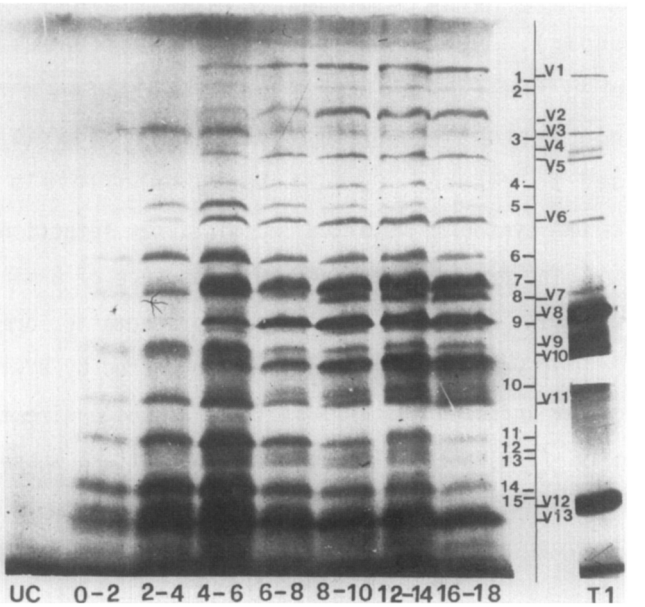

Fig. 2 Autoradiographic patterns of polypeptide synthesis in UV-irradiated *E.coli* B before (UC) and after infection with wild-type T1. Cells were pulse labelled with 10 μCi per ml of [^{35}S] for 2 min. The time after infection at which the pulses were started is specified beneath each pattern. T1 indicates the migration pattern of radioactive phage particles. Cell extracts were prepared and electrophoresed on a slab gel of 20% acrylamide as described under Materials and Methods. The origin of electrophoresis is at the top.

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tial appearance of perhaps three groups of polypeptides that were differentiated according to the period of the infective cycle during which they were synthesized. Class I comprises three proteins (Bands 3,8 and 15) that appeared immediately after infection and whose production was shut off roughly in coincidence with the onset of phage DNA replication. Synthesis of Class II proteins also started without delay and continued until lysis.Class II polypeptides are represented by bands 5, V6,6,V9,V11,11,14,V12 and V13 of Fig.3. Synthesis of Class III proteins was timed around or slightly after onset of phage DNA replications and proceeded, generally with a steady or increasing rate, until the end of the latent period. Class III, as well as Class II, includes both non structural (Bands 1,2,4,7,9,10,12,13) and structural T1 proteins (bands V1-2-3-4-5-7-8-10) , which are identified by comparison with the migration pattern of the phage polypeptides (Fig.3,T1). The sum of the mo-

lecular weight of Class I , II and III polypeptides is about 1.2×10^6 daltons or approximately 70% of the coding capacity of T1 DNA.

Search for precursors to T1 structural polypeptides. Protein processing has been found to be involved in the morphogenesis of several complex bacterial viruses including T4 (9,16-18), λ (19,20), T5 (21) and P2 (22).The stability of post-replicative T1 polypeptides whose assessed by electrophoretic analysis of proteins in samples from pulse-chase experiments initiated 9 min after infection of UV-irradiated cells . The autoradiographic pattern of the samples processed immediately after the 3 min labelling period is shown in Fig. 4 A,a.

Most radioactive proteins can be identified as phage structural components although a few rather intense bands are missing in the mature virus. Prominent among these is band 7 of molecular weight 36,500. During the chase period however this polypeptide almost disappeared while the intensity of protein V8 (molecular weight about 30,000 daltons) increased simultaneously (Fig. 4 A,bcd). Quantification of the increase of V8 relative to the diminution of 7 has been performed by assessing the peak size of the two bands.

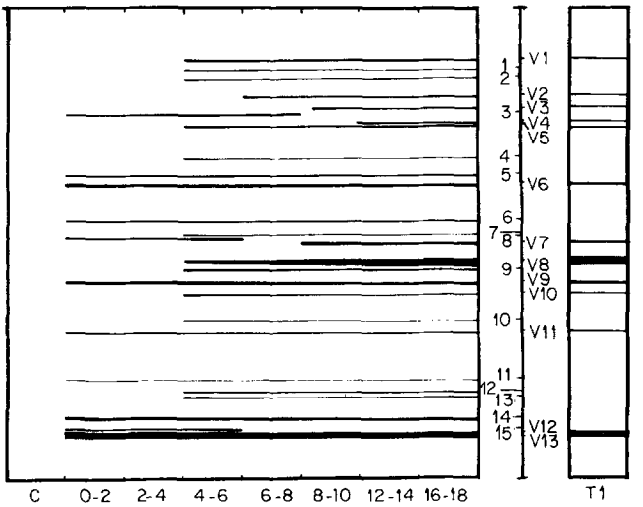

Fig. 3 Time course of T1-directed protein synthesis after infection of UV-irradiated *E.coli* B. This figure is a schematic representation of the actual autoradiogram of Fig. 2. Proteins are arranged in order of decreasing molecular weight. Lines labelled V correspond to the 13 T1 structural proteins.

As shown in Fig. 4 B the rate of disappearance of 7 is the same as the rate of appearance of V8. Furthermore, the sum 7+V8 becomes constant, as expected if 7 is converted to V8. These data clearly indicate that protein 7 is cleaved to V8. A rapid rate of precursor-to-product conversion can be inferred from the fact that during the 3 min pulse large amounts of V8 have already been produced. It is likely that other cleavages occur late in T1 growth cycle especially among the small-sized polypeptides (see for example the fate of band 14 in Fig. 4A,a).

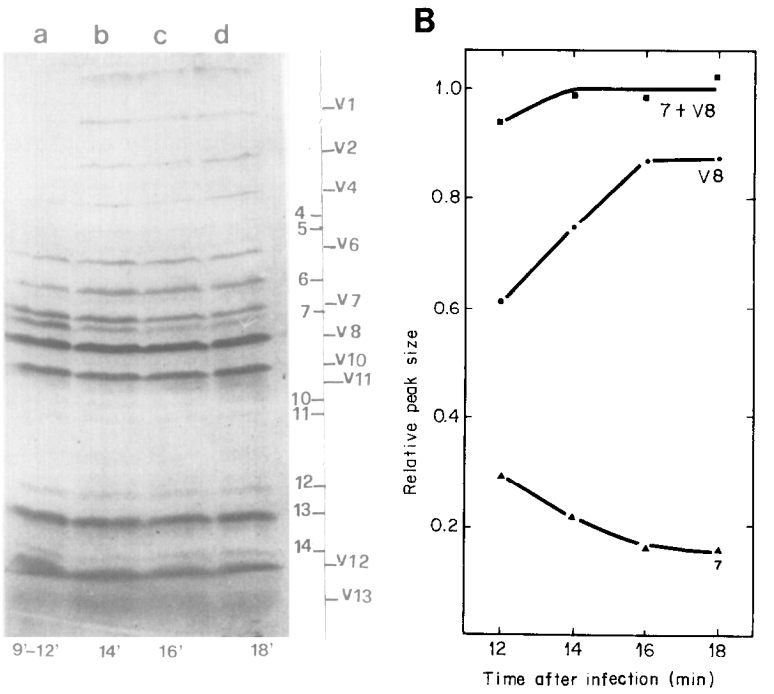

Fig. 4 A: Change of the electrophoretic mobility of some T1-directed proteins. Virus infected UV-treated cells were pulse-labelled between 9 and 12 min after the beginning of phage growth. The radioactivity was chased with an excess of *cold* precursors as specified under Materials and Methods. Samples were withdrawn 0 (a), 2 (b), 4 (c) and 6 (d) min after the end of the pulse and extracts were prepared and electrophoresed as described in the legend of Fig.2.

B: Kinetics of cleavage of polypeptide 7 to V8. The peak areas of proteins 7 and V8 at different times after the chase were determined (7,12) by scanning with a Kipp-Zenen DD2 microdensitometer the autoradiogram of Fig. 4A. The relative peak size was plotted versus the time at which the sample was taken.

DISCUSSION

T1 particles are constructed from at least 13 different protein subunits. Since no attempt has been made to separate the various structures that make up the virion no indication as to allocation of any such subunits in the molecular anatomy of T1 can be drawn at present. A notable feature of the molecular weight distribution of T1 structural components seems to be the large prevalence of relatively small-sized polypeptides (under 40,000 daltons) that account for over 80% of the phage protein. Furthermore, approximately 30% of the subunits have a molecular weight of less than 20,000 daltons. Whether this indicates a particular abundance of internal proteins (26) remains to be established.

After infection of the host , T1 directs the synthesis of at least 28 polypeptides. Based on the time of appearance T1 proteins have been distinguished into three classes,namely Class I,II and III. Little is known about the control mechanism responsible for the sequential synthesis of these groups of proteins, the selective initiation and switching-off of their production and the maintenance of the differential rates of synthesis observed with some polypeptides. It is likely however that T1,as other complex viruses,might exert this control at the trascriptional and/or the translational level.If transcriptional regulation occurs, this might prove to be similar to that described for T4 (23) and T5 (24) rather than for T3, T7 (1) and N4 (25) coliphages since the host RNA-polymerase is required throughout the lytic cycle for RNA-synthesis during T1 infection (manuscript in preparation). As far as control mechanisms are concerned it should be stressed that synthesis of T1 structural proteins is not coordinately induced. In fact, some of these polypeptides (bands V6-9-11-12-13) are produced, together with other Class II proteins,immediately after infection while synthesis of the remaining subunits(pertaining to Class III)occurs in coincidence with the onset of phage DNA replication. Analysis of the protein patterns of DNA-negative *amber* mutants (3,5) will be needed to ascertain whether synthesis of T1 structural subunits is only partially or completely independent of phage DNA replication.

Finally a complex pattern of T1 morphogenesis can be anticipated on the basis of the existence of one or more precursors that, possibly through proteolytic cleavage, give rise to the final building blocks of the phage architecture.

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REFERENCES

1. Hausmann,R. (1973), Ann. Rev.Microbiol., 27, 51-67
2. Michalke,W. (1967),Mol. Gen. Genet., 99, 12-33
3. Dexler,H. (1970), Proc. Nat. Acad.Sci. U.S.,66, 1083-1088
4. Male,C.J. and Christensen,J.R. (1970), J. Virol.,6, 727-737
5. Figurski,D.H. and Christensen, J.R. (1974), Virology, 59, 397-407
6. Bolle, A., Epstein,R.,Salser, W. and Geiduschek, E.P. (1968), J. Mol. Biol., 31, 325-348
7. Giraldi,M., Toni,M. and Schito, G.C. (1973), Virology, 55, 476-482
8. Hosoda,J. and Levinthal,C. (1968), Virology, 34, 709-727
9. Laemmli, U.K., (1970), Nature, 227, 680-685
10. Blattler,D.P., Garner,F., Van Slyke, K. and Bradley,A. (1972), J. Chromatogr., 64 , 147-155
11. Fairbanks,G.,Levinthal,C., and Reeder,R.H., (1965), Biochem.Biophys. Res. Commun., 20, 393-399
12. Adolph,K.W. and Haselkorn,R. (1972), Virology, 47, 701-710
13. Weber,K. and Osborn,M., (1969), J. Biol. Chem., 244, 4406-4412
14. Shapiro, A.L., Vinuela,E. and Maizel,J.V.(1967), Biochem. Biophys. Res. Commun., 28, 815-820
15. Mac Hattie,L.A., Rhoades,M. and Thomas,C.A.(1972), J.Mol.Biol., 72, 645-656
16. Dickson, R.C., Barnes,S.L. and Eiserling,F.A. (1970), J.Mol.Biol., 53, 461-474
17. Hosoda,J. and Cone,R., (1970), Proc. Nat. Acad. Sci. U.S.,66, 1275-1281
18. Kellenberger,E. and Kellenberger Van der Kamp,G., (1970), Fed. Enz. Biol. Soc. Lett., 8 , 140-144
19. Murialdo, H. and Siminovitch,L., (1972), Virology, 48, 785-823
20. Hendrix, R.W. and Casjens,S.R. (1974), Proc.Nat. Acad.Sci.U.S., 71, 1451-1455
21. Zweig,M. and Cummings,D.J., (1973), Virology, 51, 443-453
22. Lengyel, J.A., Goldstein, R.N., Marsh,M., Sunshine, M.G. and Calendar, R., (1973), Virology, 53, 1-23
23. Haselkorn,R., Vogel,M. and Brown,R.D. (1969), Nature, 221, 836-838
24. Beckman,L.D., Witonsky,P. and McCorquodale,D.J. (1972),J. Virol.,10, 179-186
25. Schito,G.C.,Giraldi,M. and Toni, M. (1974), Nature, 251, 340-342
26. Eiserling,F.A. and Dickson, R.C. (1972), Ann. Rev. Biochem.,41, 467-502