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LPP-1 Infection of the Blue-Green Alga *Plectonema boryanum*

II. Viral Deoxyribonucleic Acid Synthesis and Host Deoxyribonucleic Acid Breakdown

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Host and viral deoxyribonucleic acid (DNA) metabolism in LPP-1-infected *Plectonema boryanum* was studied by equilibrium centrifugation in CsCl gradients. Approximately 50% of the host DNA is degraded to acid-soluble material between 3 and 7 hr after infection. Most of the acid-soluble product is reincorporated into viral DNA. Incorporation of exogenous ^{3}H-adenine into viral DNA can be detected very early after infection (within the first 2 hr), but the bulk of viral DNA synthesis occurs between 6 and 8 hr. Both the breakdown of host DNA and the synthesis of viral DNA require protein synthesis during the first few hours of infection.

The physical properties of LPP-1 deoxyribonucleic acid (DNA) have been studied by Luftig and Haselkorn (5) and by Goldstein and Bendet (4). The DNA is double-stranded, linear, and has a contour length of about 13 μm. It has an $S_{20,w}$ of 33 and a molecular weight of about 27×10^6. According to Luftig, the buoyant density is 1.714 g/ml. In a review of the DNA of many blue-green algal species, Edelman et al. (2) found a buoyant density for *Plectonema boryanum* DNA of 1.707 g/ml. With this information about the buoyant densities of the viral and host DNA species, it appeared that it would be possible to resolve the two in equilibrium density gradients. This would enable us to answer the following questions. (i) When is the viral DNA replicated, and where does the progeny DNA originate? (ii) What effect does infection have on host DNA? The two DNA species can be resolved, and, by using the analytical ultracentrifuge, it was found that the bulk of the LPP-1 DNA is synthesized between 6 and 10 hr after infection. This was confirmed by using preparative CsCl equilibrium centrifugation of DNA labeled during viral infection.

We have also found that the host DNA is degraded very soon after infection, until by 6 or 7 hr about half is broken down. Much of these breakdown products are then incorporated into viral DNA. Both viral DNA synthesis and host DNA breakdown are prevented by chloramphenicol, indicating that both processes require the synthesis of new proteins.

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MATERIALS AND METHODS

Algae and virus. The growth conditions of the alga *P. boryanum* and isolation procedures for the virus, LPP-1, are described in an accompanying paper (9).

Radioactive labeling of DNA. ^{14}C- or ^{3}H-labeled compounds were diluted to give a final specific activity of 5 to 200 mCi/mmole in Chu no. 10 medium. Radioactivity was determined by adjusting a sample containing 10^6 to 10^7 cells/ml to 0.5 N in NaOH and incubating for 16 to 18 hr at 37 C. An equal volume of 10% trichloroacetic acid was then added, and the precipitate was collected by filtration on membrane filters (HA; Millipore Corp., Bedford, Mass.) and washed with 15 ml of 5% trichloroacetic acid, followed by 5 ml of 80% ethanol.

^{14}C was counted in a Beckman Low-Beta II gas flow counter at an efficiency of 25%. For tritium, the filters were placed in scintillation vials, heated at 60 C for 1 to 2 hr, and covered with 4 to 8 ml of scintillation fluid (Liquifluor). Samples were counted in a Packard Tri-Carb Scintillation Counter at 20 to 30% efficiency.

Chloramphenicol (CAP) was a gift from Parke, Davis & Co. The effect of CAP on protein synthesis is described in an accompanying paper (10).

DNA extraction and analysis. DNA from virus or from infected cells was isolated by a combination of the Marmur procedure (8) and phenol extraction (12). Cells were spun down and resuspended in saline ethylenediaminetetraacetic acid and treated with lysozyme. The lysozyme treatment varied from 100 μg/ml for 12 hr at 4 C to 1 mg/ml for 2 to 3 hr at

37 C. This solution was brought to 2% sodium dodecyl sulfate and 50 μg of ribonuclease per ml; it was incubated at room temperature for 1 to 2 hr. The DNA was extracted three times with phenol [saturated with 0.1 M tris(hydroxymethyl)aminomethane, unbuffered], dialyzed for 6 hr against 1 M NaCl, 0.01 M PO_4 (*p*H 6.8) and, finally, against three changes of 0.01 M PO_4 (*p*H 6.8).

Analytical CsCl ultracentrifugation was performed in a Beckman model E ultracentrifuge according to Mandel et al. (7). The technique of Szybalski (11) was used for four cell runs. Photographs were traced with a Joyce-Loebl densitometer.

Preparative CsCl centrifugation was carried out in a Spinco 40 rotor by the method of Flamm et al. (3). By adjusting the initial density to 1.715 to 1.720 g/cm^2 so that *P. boryanum* DNA banded in the upper part of the gradient, excellent resolution was obtained. The gradients were run for 70 hr at 33,000 rev/min and 25 C; 5-to 9-drop fractions were collected. The absorbancy of each fraction was measured at 260 nm after addition of 0.5 ml of 0.01 M PO_4 (*p*H 6.8). Each fraction was then precipitated with trichloroacetic acid and collected on filters for liquid scintillation counting. After the results were plotted and the base line was drawn freehand, the area under each peak was estimated by weighing a traced replica.

RESULTS

Analytical ultracentrifugation. We first wished to determine whether the difference of 0.007 g/ml between LPP-1 DNA and *P. boryanum* DNA was sufficient to permit quantitative determination of the two DNA species in mixtures. By using the DNA from the *Bacillus subtilis* virus SP01 as reference (ρ = 1.742), we found the buoyant density of LPP-1 DNA to be 1.713 ± 0.002, in agreement with Luftig and Haselkorn (5), and that of *P. boryanum* to be 1.706 ± 0.002, in agreement with Edelman et al. (2). In some experiments, a minor component was found at ρ = 1.730 in preparations of algal DNA. This component is not present in cultures rigorously freed of bacteria. It does not affect the analyses presented below.

Mixtures of LPP-1 and *P. boryanum* DNA were prepared with relative proportions based on absorbancy at 260 nm. These mixtures were then analyzed by analytical ultracentrifugation in CsCl gradients. One such mixture is shown in Fig. 1 in which 0.2 μg of *P. boryanum* DNA was added to 1.0 μg of LPP-1 DNA. The two DNA species are readily resolved; when the densitometer scans were cut out and weighed, it was found that the *Plectonema* DNA was 15% of the total. This is in good agreement with the expected 16%, and shows that this technique is sensitive enough for our purposes.

It should be mentioned that the A_{260} to A_{230} ratio was consistently higher later in infection

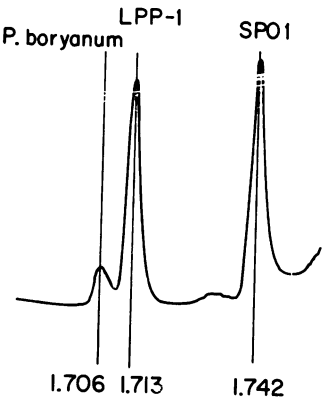

FIG. 1. *Sedimentation equilibrium in a CsCl density gradient of a mixture of 0.2 μg of P. boryanum DNA and 1.0 μg of LPP-1 DNA. Centrifugation was at 25 C for 24 hr at 44,000 rev/min. The marker is the B. subtilis phage SP01 DNA.*

than in uninfected or 2-hr infected cells. The polysaccharide band which can be seen in the CsCl gradients at a density of 1.658 g/ml (2) was not found in DNA prepared from cells later in infection (greater than 4 hr). This probably means that the polysaccharide of the cell wall is broken down during infection.

Having established that the relative proportions of host and viral DNA could be determined accurately in mixtures, we then determined that ratio as a function of time after LPP-1 infection. DNA prepared from cells 2 hr after infection is shown in Fig. 2a. At a multiplicity of infection (MOI) of 10, assuming a molecular weight of 28 × 10^6 for LPP-1 and 2 × 10^9 for *P. boryanum* DNA, LPP-1 DNA should account for 12% of the total DNA. We find a ratio of 11%, in reasonable agreement with this expectation. In Fig. 2b and 2c, the DNA prepared 4 and 6 hr after infection, respectively, is shown and the relative proportion of LPP-1 DNA has increased slightly to 16 and 19%, respectively. We shall see below that this increase is due predominantly to host DNA breakdown, although there is significant incorporation of ^{3}H-adenine into LPP-1 DNA during the first 6 hr of infection.

Net viral DNA synthesis is clearly observed by 7th hr after infection (Fig. 2d). LPP-1 DNA has now increased to 38% of the total. By 8 hr (Fig. 2e), the relative concentration of LPP-1 DNA has become 73%, by 10 hr, 84%, and by 12 hr, 86% (Fig. 2f, 2g). These results are summarized in Fig. 3a. If we assume that 50% of the host DNA (molecular weight = 2 × 10^9) is broken down, 86% LPP-1 DNA would yield about 300 viral DNA equivalents per cell or 30 per infecting

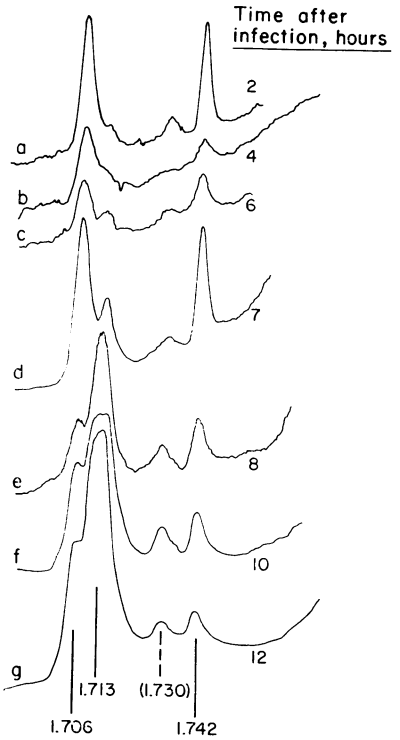

FIG. 2. *Sedimentation equilibrium in CsCl density gradients of DNA extracted from LPP-1-infected P. boryanum (MOI = 10) at various times after infection. Centrifugation was at 25 C for 24 hr at 44,000 rev/min. The DNA species from 2, 4, 6, and 8 hr were centrifuged in a four-cell AN-F rotor; the others, in a AN-D rotor.*

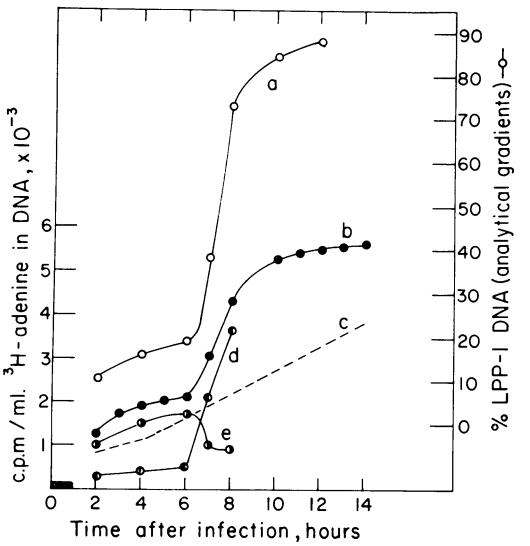

FIG. 3. *DNA synthesis in LPP-1 infection. (a) Summary of CsCl equilibrium centrifugation experiment. The percentage of LPP-1 DNA at each time was determined from the areas under the host and viral DNA bands in Fig. 2. (b) and (c) Cumulative incorporation of ^{3}H-adenine into DNA. Cells were infected at MOI = 10 and kept in the dark, 1 hr; ^{3}H-adenine was then added at 0.5 μCi/ml, 100 mCi/mmole. (b) Infected cells, (c) uninfected cells. The incorporation into infected cells, curve b, was divided into a viral DNA component (d) and a host DNA component (e) by using the data from the experiment shown in Fig. 4 and 5 to compute the proportional synthesis at each time.*

virus by 12 hr. If this DNA were packaged into infectious particles, we would have a titer of 3×10^{10} plaque-forming units (PFU)/ml at 12 hr. The observed titer was 1.7×10^{10} PFU/ml which implies that about 55% of the viral DNA is in assembled infectious particles at 12 hr.

Labeling of P. boryanum with nucleic acid precursors. No systematic study of radioisotope incorporation into blue-green algal nucleic acids has been reported. We therefore studied the incorporation of several nucleic acid precursors, the results of which are listed in Table 1. Adenine and guanine are incorporated well into both DNA and ribonucleic acid (RNA), whereas uracil is incorporated very well into RNA. With the exception of adenosine, the nucleosides are incorporated poorly. Orotic acid is incorporated only after a 6-hr lag and then at about the same rate as adenosine. Uridine and thymidine are incorporated very poorly None of the compounds studied was found to be incorporated specifically into DNA. Therefore, from the results of this

TABLE 1. *Incorporation of nucleic acid precursors into P. boryanum*[a]

Precursor	Relative incorporation into[b]	
	DNA	RNA
Guanine	++++	++++
Adenine	++++	++++
Adenosine	++	++
Uracil	++	++++
Uridine	—	—
Orotic acid	+	+
Thymine	+	+
Thymidine	—	—

[a] Uninfected *P. boryanum* was centrifuged at 5,000 rev/min for 5 min and resuspended in fresh Chu no. 10 medium 12 hr before the addition of label. The precursors were added at a concentration of 1 μCi/ml and a specific activity of 30 to 50 mCi/mmole.

[b] Symbols: ++++, incorporation of 50% of the total radioactivity in the medium by 12 hr; —, less than 1% incorporation at 12 hr.

experiment, we chose to pulse-label infected cells with adenine and to determine incorporation into DNA after removal of RNA by alkaline hydrolysis.

Incorporation of ^{3}H-adenine into LPP-1 DNA-pulse label: preparative CsCl sedimentation equilibrium. Cumulative incorporation of ^{3}H-adenine into DNA in an infected culture of *P. boryanum* is shown in Fig. 3b. There is some incorporation between 2 and 6 hr and then a massive increase between 6 and 8 hr. The distribution of ^{3}H-adenine in host and viral DNA at various times after infection was determined by preparative ultracentrifugation in CsCl gradients (Fig. 4a, 4b). ^{3}H-adenine, introduced between 1 and 2 hr after infection, is incorporated predominantly into host DNA, but there is some incorporation into LPP-1 DNA as well (Fig. 4a). The same is true for pulses from 2 to 4 and 4 to 6 hr after infection. However, in the pulse between 6 and 7 hr, the label goes preferentially into LPP-1 DNA. This is also true of the 7- to 8-hr pulse (Fig. 4b). It is significant that as late as 7 hr after infection, label still enters host DNA, because half of the host DNA is degraded to acid-soluble material by the 6th hr after infection (*see below*).

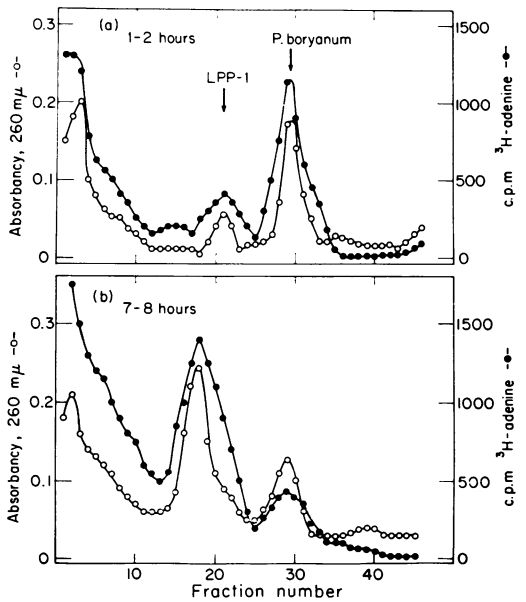

Fig. 4. Equilibrium centrifugation of ^{3}H-labeled DNA from LPP-1-infected P. boryanum. Six hundred milliliters of P. boryanum at 10^8 cells/ml was infected with LPP-1 at MOI = 10. At 2, 4, 6, 7, and 8 hr after infection, 100-ml amounts were removed and added to 50 μCi of ^{3}H-adenine. Incorporation was terminated by addition of $CHCl_3$ and chilling to 0 C. Each DNA preparation was centrifuged in a Spinco 40 rotor at 25 C for 70 hr at 33,000 rev/min. Each tube contained 4.5 ml of solution with an average density of 1.715 to 1.720 g/ml. Only the earliest and latest DNA samples are shown here. Data from all five gradients are shown in Fig. 5.

The data on ^{3}H-adenine incorporation into LPP-1 and *P. boryanum* DNA are summarized in Fig. 5. By using these results on the distribution of label between host and viral DNA, we can redraw the incorporation curve in Fig. 3 as the sum of two components (Fig. 3d, 3e). Incorporation into *P. boryanum* DNA increases until 6 hr after infection and then drops. Incorporation into viral DNA is constant until the 6th hr, at which time abundant viral DNA synthesis begins.

P. boryanum DNA breakdown. To determine the fate of host DNA after LPP-1 infection, a culture of *P. boryanum* was labeled with ^{3}H-adenine for one generation (24 hr), which was followed by one-generation chase with unlabeled adenine. These cells were then infected, and samples were removed for measurement of alkali-stable, trichloroacetic acid-precipitable radioactivity. Two typical experiments are shown in the controls of Fig. 9a and b. There is a small amount of breakdown while the culture is in the dark (2 hr). However, between 2 and 3 hr after infection, host DNA begins to be degraded, and by the 7th hr this process has reached a maximum. The breakdown products are then reincorporated into viral DNA. This experiment only shows the gross difference between the amount synthesized and the amount broken down, so it is not possible to tell if the minimum of the controls in Fig. 9a and b reflects the total amount

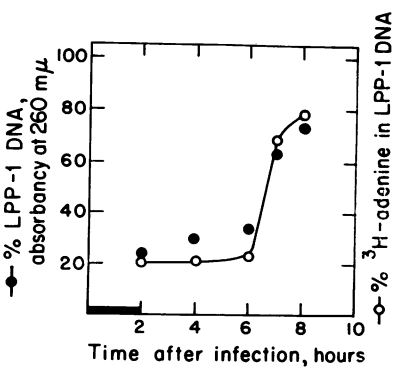

FIG. 5. *DNA synthesis in LPP-1-infected P. boryanum. Both radioactive and ultraviolet absorbancy peaks in each preparative gradient from the experiment shown in Fig. 4 were measured and the proportion of LPP-1 DNA was calculated. The times indicate when the radioactive pulse was terminated and the DNA was prepared.*

broken down or just the point at which the amount of newly synthesized DNA becomes greater than the amount broken down.

To see what happened to the breakdown products, another host-label experiment was performed; the DNA was prepared at 0, 7, 10, and 14 hr, and this DNA was centrifuged on preparative CsCl gradients. The results of this experiment are shown in Fig. 6 and 7. At 7 hr (Fig. 7a), a substantial amount of host breakdown product has been incorporated into LPP-1 DNA. By 10 hr, LPP-1 DNA synthesis, as measured by absorbancy at 260 nm, has increased greatly, but the incorporation of labeled breakdown products has increased only slightly. At 14 hr, the amount of label in the LPP-1 DNA has increased to about 55% of the total.

In this experiment, we also tried to determine whether any *P. boryanum* DNA is incorporated intact into LPP-1 particles. To do this, we allowed infection to continue until 24 hr. We then treated the culture with deoxyribonuclease, collected the mature virus by centrifugation, and purified it twice on CsCl step gradients. The DNA, prepared as usual, was run on a preparative CsCl gradient (Fig. 6b). The major peak of LPP-1 DNA can be seen at fraction 25; host DNA would be found at fractions 35 to 36 (Fig. 6a). Although there is a very slight peak of radioactivity at fraction 36, there is no absorbancy in that fraction, and we conclude that there is either no host DNA carried in phage particles (less than 0.1%) or that, if there is, it is integrated with the phage genome.

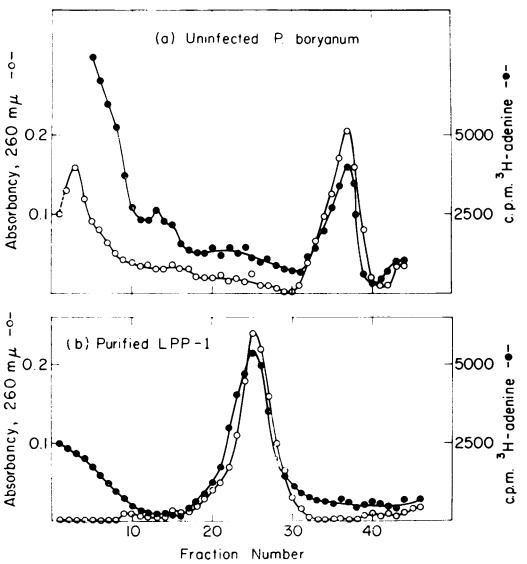

FIG. 6. *Absence of intact host DNA from purified LPP-1. (a) P. boryanum was labeled with ³H-adenine (0.5 μCi/ml, 100 mCi/mmole) for one generation (24 hr), chased for another generation with 100-fold excess of unlabeled adenine, and then infected with LPP-1 at MOI = 10. DNA was prepared from a sample of cells prior to infection and run in a CsCl gradient as described in Fig. 4. (b) LPP-1 was harvested 24 hr after infection of cells previously labeled with ³H-adenine. DNA was extracted from purified virus and run in CsCl as described in Fig. 4.*

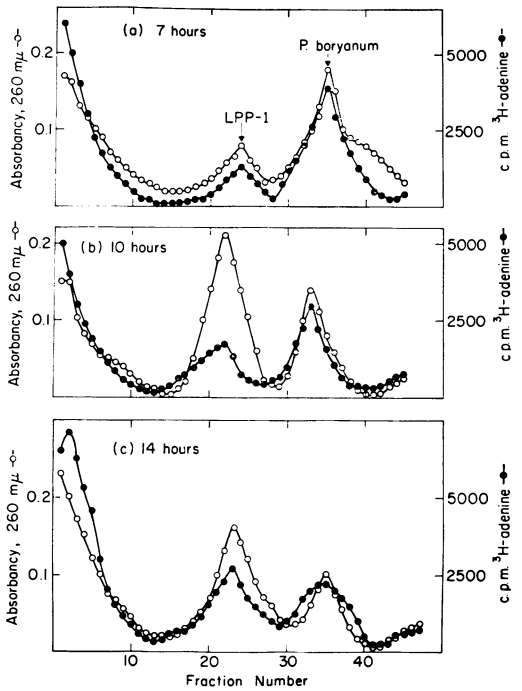

FIG. 7. *Incorporation of host DNA breakdown products into LPP-1 DNA. From the experiment described in Fig. 6a, 100-ml samples were taken at 7, 10, and 14 hr for preparation of total DNA. Centrifugation was in CsCl as described in Fig. 4.*

Effect of CAP on LPP-1 DNA synthesis and host breakdown. Protein synthesis shortly after infection is required for both host DNA breakdown and viral DNA synthesis. The approximate times of synthesis of the relevant proteins can be determined by using CAP to inhibit protein synthesis. The effect of CAP on viral DNA synthesis is shown in Fig. 8. The addition of CAP at 2 or 4 hr does not permit more than 1 hr of DNA synthesis, and most of this DNA is eventually degraded.

When CAP is added at 6 hr, there is net incorporation for 4 more hr, which is followed by some degradation. Addition at 8 hr shows no change from the control until 11 hr, when degradation occurs once more. This experiment

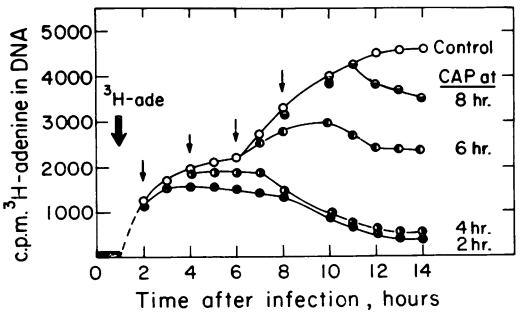

FIG. 8. *Effect of CAP on the cumulative incorporation of ^{3}H-adenine into DNA in LPP-1-infected P. boryanum. Infection was at MOI = 10; 0.5 μCi of ^{3}H-adenine per ml was added after 1 hr of incubation in the dark. CAP at 100 μg/ml was added to portions of the infected culture at times indicated by the arrows.*

shows that the synthesis of protein is necessary for both the replication of LPP-1 DNA and its stabilization. Proteins needed for abundant replication are synthesized predominantly between 4 and 6 hr after infection. The degradation of some newly synthesized LPP-1 DNA 3 to 4 hr after the addition of CAP late in infection probably reflects the fate of unpackaged viral DNA.

The breakdown of host DNA requires protein synthesis during the first 3 hr after LPP-1 infection. This requirement was demonstrated in an experiment in which CAP was added at various times after LPP-1 infection of cells whose DNA had been labeled prior to infection. Uninfected cells, treated with CAP or not, showed no change in alkali-stable, trichloroacetic acid-precipitable radioactivity. When CAP was added at the same time as the virus to the cells (Fig. 9), there was very little breakdown of host DNA; this remained constant until 14 hr. Addition of CAP at 1 hr allowed over 50% of the host DNA breakdown to occur, whereas addition at 2 hr permitted 75% DNA degradation (Fig. 9a). CAP addition at 3 hr permitted host DNA breakdown below the control, also preventing later reincorporation (Fig. 9b). Addition of CAP at 4 hr (Fig. 9a) or at 5 hr (Fig. 9b) allowed nearly maximum host DNA breakdown but also permitted some reincorporation. Therefore, during the first 3 hr after infection, all of the proteins required for host DNA degradation are synthesized, leading to breakdown of half the host DNA by 7 to 8 hr. We refer to this ability of a cell to perform a certain function at a given time as "capacity." The capacity of infected *P. boryanum* for host DNA breakdown at 3 hr is 100%, whereas, at the same time, the capacity for LPP-1 DNA synthesis is only a few per cent.

A compilation of capacity measurements is presented in Fig. 10. The proteins required for host DNA degradation are completely synthesized by the 3rd hr of infection, and the host DNA degradation occurs mainly from 2 to 8 hr. The invagination of the photosynthetic lamellae followed similar kinetics about 1 hr later (9). The LPP-1 DNA replicating enzyme(s) is present early at a low level, until the 6th hr, when a large

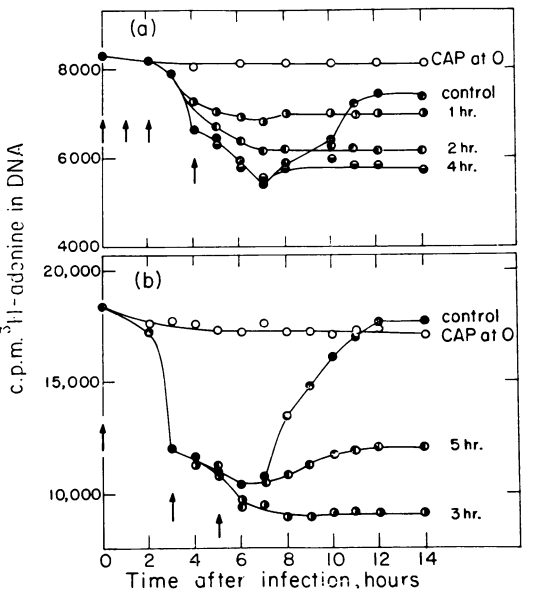

FIG. 9. *Effect of CAP on the breakdown of P. boryanum DNA. P. boryanum was grown in the presence of 0.5 μCi of ^{3}H-adenine per ml for one generation, chased with unlabeled adenine for one generation, and then infected at MOI = 10. The arrows refer to the time of addition of CAP. In both experiments, uninfected controls (± CAP) showed no decrease in alkali-stable, trichloroacetic acid-precipitable radioactivity*

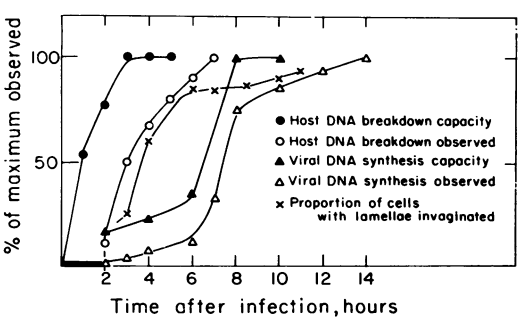

FIG. 10. *The time course of early functions in LPP-1 infection. Data for host DNA breakdown and viral DNA synthesis are taken from Fig. 3, 5, 8, and 9. The quantitative electron microscope data are from Sherman and Haselkorn (9).*

amplification occurs. The important conclusion from the CAP experiments is that the synthesis of proteins for host DNA degradation and viral DNA synthesis occurs at different times, so there are at least two distinguishable periods of protein synthesis prior to viral DNA replication.

DISCUSSION

We have shown that viral DNA is synthesized predominantly between 6 and 8 hr after infection, although there is significant incorporation of labeled precursor into viral DNA during the first 6 hr. This process requires viral-directed protein synthesis during the first 6 hr. About 50% of the host DNA is broken down and most of the breakdown products are reincorporated into viral DNA. However, incorporation into host DNA is not shut off completely—residual incorporation continues until the 8th hr. Host DNA breakdown is also dependent on viral-directed protein synthesis. The degradative activity is completely synthesized by 3 hr, whereas the breakdown reaches a maximum by 7 hr.

The incorporation of ^{3}H-adenine into viral DNA early in infection, prior to the abundant DNA synthesis beginning at the 6th hr, cannot be explained entirely by precocious synthesis in an asynchronously infected population. After 4 hr, for example, there is less than one complete virus particle per cell, and all of the increase in the relative proportion of viral DNA measured in density gradients can be attributed to host DNA breakdown. At this time, however, incorporation into viral DNA is about 10% of that observed at the peak of viral DNA synthesis. Two possible sources of this active pre-replication incorporation of adenine are recombination and repair. Recombination is likely because these experiments are done at high multiplicity of infection (10). Repair is suggested because blue-green algae are very resistant to both ultraviolet and X rays and contain very active repair systems (13).

Our inability to find intact host DNA in viral particles (Fig. 6) is disappointing because we had hoped to use LPP-1 or its relatives for transduction. The rather extreme compartmentalization evident in the electron micrographs of the preceding paper (9) suggests a reason for this discrimination: viral assembly occurs in the virogenic stroma which is separated from the nucleoplasm of the host by the system of photosynthetic lamellae, and the only access to viral capsids for host DNA is through the soluble nucleotide pool.

LPP-1 is superficially similar to the coliphage T7 in a number of ways (6). This similarity is quite extensive at the level of viral proteins, as described in the next paper (10). The similarities in DNA metabolism are also quite striking. T7 induces several nucleases whose combined action solubilizes about 50% of the host DNA. One nuclease, which has been purified, acts on both single- and double-stranded DNA (1). In vivo, the reincorporation into viral DNA of the products of degradation of host DNA is so rapid that no decrease in total acid-precipitable material can be detected. However, in nonpermissive infection with mutants in the T7 DNA polymerase, there is no reincorporation of host material. In that case, the kinetics of degradation of host DNA are similar to those observed in LPP-1 infection, taking into account the difference in overall growth rates in the two systems.

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LITERATURE CITED

1. Center, M. S., F. W. Studier, and C. C. Richardson. 1970. The structural gene for a T7 endonuclease essential for phage DNA synthesis. Proc. Nat. Acad. Sci. U.S.A. 65: 242–248.
2. Edelman, M., D. Swinton, J. A. Schiff, H. T. Epstein, and B. Zeldin. 1967. Deoxyribonucleic acid of the blue-green algae (*Cyanophyta*). Bacteriol. Rev. 31:315–331.
3. Flamm, W. G., H. E. Bond, and H. E. Burr. 1966. Density-gradient centrifugation of DNA in a fixed angle rotor. A higher order of resolution. Biochim. Biophys. Acta 129: 310–319.
4. Goldstein, D. A., and I. J. Bendet. 1967. Physical properties of the DNA from the blue-green algal virus LPP-1. Virology 32:614–618.
5. Luftig, R., and R. Haselkorn. 1967. Morphology of a virus of blue-green algae and properties of its deoxyribonucleic acid. J. Virol. 1:344–361.
6. Luftig, R., and R. Haselkorn. 1968. Comparison of blue-green algae virus LPP-1 and the morphologically related viruses Gm and coliphage T7. Virology 34:675–678.
7. Mandel, M., C. L. Schildkraut, and J. Marmur. 1968. Use of CsCl density gradient analysis for determining the guanine plus cytosine content of DNA, p. 184–195. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. 12, part B. Academic Press Inc., New York.
8. Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. 3:208–218.
9. Sherman, L. A., and R. Haselkorn. 1970. LPP-1 infection of the blue-green alga *Plectonema boryanum*. I. Electron microscopy. J. Virol. 6:820–833.
10. Sherman, L. A., and R. Haselkorn. 1970. LPP-1 infection of the blue-green alga *Plectonema boryanum*. III. Protein synthesis. J. Virol. 6:841–846.
11. Szybalski, W. 1968. Use of cesium sulfate for equilibrium density gradient centrifugation, p. 330–360. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. 12, part B. Academic Press Inc., New York.
12. Thomas, C. A., Jr., and J. Abelson. 1966. The isolation and characterization of DNA from bacteriophage, p. 553–561. *In* G. L. Cantoni and D. R. Davies (ed.), Procedures in nucleic acid research. Harper and Row, New York.
13. Wu, J. H., R. A. Lewin, and H. Werbin. 1967. Photoreactivation of UV-irradiated blue-green algal virus LPP-1. Virology 31:657–664.