Electron Microscopy of the Infection Process of the Blue-Green Alga Virus

KENNETH M. SMITH, R. M. BROWN, JR., P. L. WALNE

Department of Botany and The Cell Research Institute, University of Texas, Austin, Texas 78712

AND D. A. GOLDSTEIN

Department of Biophysics, University of Pittsburgh, Pittsburgh, Pennsylvania 15213

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Particles of BGAV seem to attach to the walls of host cells by a long tail, like that of some bacteriophages, and at infection they probably inject their nucleic acid into the cells in a bacteriophage-like manner. In cultures inoculated some days earlier, masses of virus particles were found in thin sections of cells of the species Plectonema boryanum, P. notatum, and Lyngbya sp. Four other species were also susceptible to infection.

INTRODUCTION

Smith et al. (1966) gave a preliminary account of the infection process of the blue-green alga virus (BGAV) showing that the most characteristic sign of infection was the lateral displacement of the photosynthetic lamellae. Newly formed virus particles were found to be closely associated with the photosynthetic lamellae and to remain attached to them after the whole cell was completely lysed. The replication process of the virus and its method of entry into the cell, however, were not elucidated.

The work described below gives further details of the behavior of the virus in cells, and indicates that the virus particles have a long tail and not, as previously suggested, a very short one (Safferman and Morris, 1963; Smith et al. 1966).

MATERIALS AND METHODS

Our previous work was done with healthy and virus-infected algae grown in liquid cultures, and on purified virus suspensions.

Studies using other techniques are now described. Instead of liquid cultures, the virus was propagated in algae growing on an agar medium, either Chu's No. 10, or Bold's basal medium (Bischoff and Bold, 1963) in Petri dishes. As soon as the plaques formed on the surface, portions of the edges of the plaques were removed and the material fixed first at room temperature for 1 hour in a mixture consisting of 3% acrolein and 3% glutaraldehyde in a cacodylate buffer, and then in 2% osmium tetroxide at room temperature for 1 hour. This was followed by staining overnight at 5° in 0.5% uranyl acetate before dehydration. Sections were cut on a Sorvall Porter-Blum Mark II ultramicrotome with a diamond knife, and were then stained first in uranyl acetate and then in lead citrate. Electron micrographs were taken with RCA models EMU-3F and EMU-3G and Siemens Elmiskop I.

Other portions of the plaques were stained with phosphotungstic acid and mounted on the grids without sectioning or fixation, or were examined by phase microscopy.

Under our conditions, plaques formed 2 days after inoculation in actively growing
cultures of Plectonema boryanum and 4 days afterward in aged cultures. All pictures of P. boryanum presented in this communication were made from 4-day infected cultures.

RESULTS

The advantages of the new fixation methods were at once apparent. The virus particles were shown to have a long "tail" more like that of some bacteriophages, and numbers of them were seen apparently attached by these "tails" to the photosynthetic lamellae and to the external cell wall (Figs. 1, 2, and 4).

Figure 2 is a section of part of a cell showing virus particles and some "ghosts" attached to the outer cell wall by their long tails. There is some suggestion that the "tails" penetrate the cell wall. In the mass of material just below the point of attachment of the particles are three bodies which may be developing virus particles (arrows).

These long tails were not observed in purified preparations of the virus and they were perhaps broken off during the process of purification used in our previous work, leaving the tail plate with a short extension of the hollow tail. Figures 1 a–c show negatively stained preparations of lysed cell debris, including the photosynthetic lamellae, with attached virus particles. Note the area of differential negative staining along the tail region; the arrows indicate sites of possible fracture in Figs. 1a and 1b. An alternative possibility is that the tails may have been retracted.

Smith et al. (1966) suggested that replication of the virus took place in the nucleoplasm of the cell, but definite evidence of this was lacking. By means of the new technique, however, more evidence on this point is forthcoming (Figs 2–4). What may be called "virogenic stromata” develop in the nuclear region of the cell, apparently closely associated with the "polyhedral bodies,” and masses of virus particles can be seen in various stages of development within the stroma (Figs. 3 and 4). The supporting evidence

![Image of virus particles and cell structures](image-url)

**Fig. 1.** Attachment of the virus particles to the photosynthetic lamellae; in (a) and (b) arrows mark the site of possible fracture in the tail; in (c) arrow marks two "ghosts." Scale is 100 µm.
Fig. 2. Section of part of a cell showing virus particles and some "ghosts" attached to the outer cell wall by their long tails, which are thought to penetrate the wall. In the mass below the cell wall are three bodies which may be developing virus particles (arrows): Compare with Fig. 3. Scale is 1 μ.
Fig. 3. Sections of two cells showing the virogenic stroma with masses of developing virus particles: Arrows mark the "polyhedral body." Scale is 1 μ.
Fig. 4. Section through parts of two cells; in the center is a virogenic stroma at an early stage; the arrow marks a developing virus particle. In the right-hand cell the arrow marks a virogenic stroma in an advanced stage. Note the ruptured photosynthetic lamellae. The third arrow marks virus “ghosts” apparently attached to the outside wall of the cell. Scale is 1 μ.
Fig. 5. Section through four cells showing progressive development of rather advanced stages of infection; the cell on the left is almost completely lysed. Arrows mark the apparent breakdown of the intervening wall. *Inset:* a virus particle attached by its tail to the intervening wall; Note the progressive breakdown of photosynthetic lamellae and the release of many virus particles to the cell periphery. Scale is 1 μ; inset, 100 μ.

Fig. 6 Photomicrograph, by phase contrast, of healthy and infected filaments of *Plectonema* that development of virus is in the nucleoplasm may be found in earlier studies (Smith *et al.*, 1966) in which an increase in Feulgen staining and aeridine orange fluorescence was noted in the central nucleoplasmic region of infected cells. The long "tail" is not visible in the particles at this stage, and it may be developed later. Figure 4 shows sections of two cells containing viroenic stromata in what we think are early and late stages of development; the arrow outside the cell marks the empty membranes or "ghosts" of virus particles still attached to the cell wall.

It is not yet clear whether virus passes from cell to cell in addition to the infection through the outside wall. However, there is a suggestion in Fig. 5 that this may take place. The inset shows a virus particle attached to the internal transverse wall of the filament.

*boryanum*; arrow marks a cell in an advanced stage of infection; debris of lysed cells in background. Scale is 5 μ.
Fig. 7. Cross section through two filaments of *Lyngbya* sp. showing the photosynthetic lamellae pushed to one side by the virogenic stroma in which are many developing virus particles. Note also some attached "ghosts." Arrows mark the folding back of the photosynthetic lamellae. Scale is 1 μ.
Fig. 8. Longitudinal section through a cell of *Lyngbya* sp.; note many virus particles and “ghosts” attached to outside wall. A few particles can be seen in the virogenic stroma. Scale is 1 μ.
We suggest that Fig. 5 represents a late stage of infection just before lysis of the wall; the photosynthetic lamellae appear to be degenerating.

Although filaments of *Plectonema boryanum* are extremely small, changes within the infected cells were detected in the optical microscope by means of fluorescent and Feulgen staining (Smith et al., 1966). Changes in the filaments can also be seen by means of phase contrast microscopy. In Fig. 6 are several filaments in various stages of infection; the arrow marks a filament which is in an advanced stage. The particles in the background are the lysed remnants of cells, mainly the photosynthetic lamellae.

We have observed many instances both of virus particles and their “ghosts” attached to the outer cell wall and to the photosynthetic lamellae. Furthermore, we think that the “tail” of the virus particle shown in Fig. 2 penetrates the cell wall.

This evidence seems to us to justify the opinion that the whole virus particle does not enter the cell as we thought possible (Smith et al. 1966), but injects its DNA into the cell in the classic manner of some phages. Confirmation of this supposition must wait on experiments using labeled virus, as first described by Hershey and Chase (1952).

**Host Range of the Blue-Green Alga Virus**

Safferman and Morris (1963) state that the BGAV infects *Lyngbya* and *Phormidium* spp. in addition to *Plectonema boryanum*.

We have studied the virus in the following blue-green algae: *Plectonema eolobrdocoides, P. notatum, Phormidium foveolarum, P. luridum var. olivaceae, Phormidium sp., Lyngbya sp.*

The overall pattern of infection seems similar in all these species. In *Lyngbya* and *Phormidium* the photosynthetic lamellae can be seen displaced in a manner similar to that which occurs in *Plectonema*. In Fig. 7 is a cross section of two filaments of *Lyngbya* sp. Note the photosynthetic lamellae are pushed to one side by the virogenic stroma in which are many virus particles. In no case has the tail been observed in these masses of differentiating virus particles (Figs. 3, 4, and 7).

In Fig. 9 is a longitudinal section of two cells of *Plectonema notatum*; note the many virus particles in the end cell.

Numerous virus particles and their “ghosts” have been observed attached to the outer wall in *Lyngbya* sp. (Fig. 8), and in
Fig. 10. (A) Virus particles and ghosts attached by their tails to the photosynthetic lamellae of the lysed cell of *Phormidium luridum*. Scale is 1 μ. (B) Virus particles arranged, probably attached by their tails, around a membrane containing a polyhedral body in *Phormidium foveolarum*. Scale is 100 μ.
Phormidium foveolarum particles have been seen associated with a membrane surrounding what appears to be a polyhedral body (Fig. 10B). Figure 10A shows the lysed remnants of cells of *P. luridem*. Virus particles and "ghosts" can be seen attached to the photosynthetic lamellae. Some of the long "tails" are visible.

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REFERENCES


