Studies of virus infection of isolated protoplasts

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INTRODUCTION

Although considerable advances have been made in recent years in our knowledge of plant viruses in general our knowledge of the early stages of the virus infection on plant cells is almost nonexistent. No model system has been available in which the cells under study can be simultaneously exposed to virus particles and become infected. As a result it has been impossible to obtain a synchrony of infection of plant cells by viruses. The usual plant tissue cultures have not contributed in any significant way to our ability to obtain synchronous infection. As pointed out by Kassanis [5] one of the major difficulties is the presence of the cell wall.

The finding that protoplasts can be readily isolated from tomato fruit locule tissue [4] has enabled a new experimental approach to be adopted in which isolated protoplasts (from which the cell wall has been removed) are incubated with virus. These isolated protoplasts have been shown to take up virus into vesicles in the cytoplasm by a process of pinocytosis [1]. It has also been shown that these isolated protoplasts readily regenerate a cell wall when incubated in suitable culture media [6]. From these experimental observations the concept arose of obtaining unaided entry of tobacco mosaic virus into isolated protoplasts, and following regeneration of the protoplasts to obtain cells which contained virus in vesicles in the cytoplasm (Fig. 1).

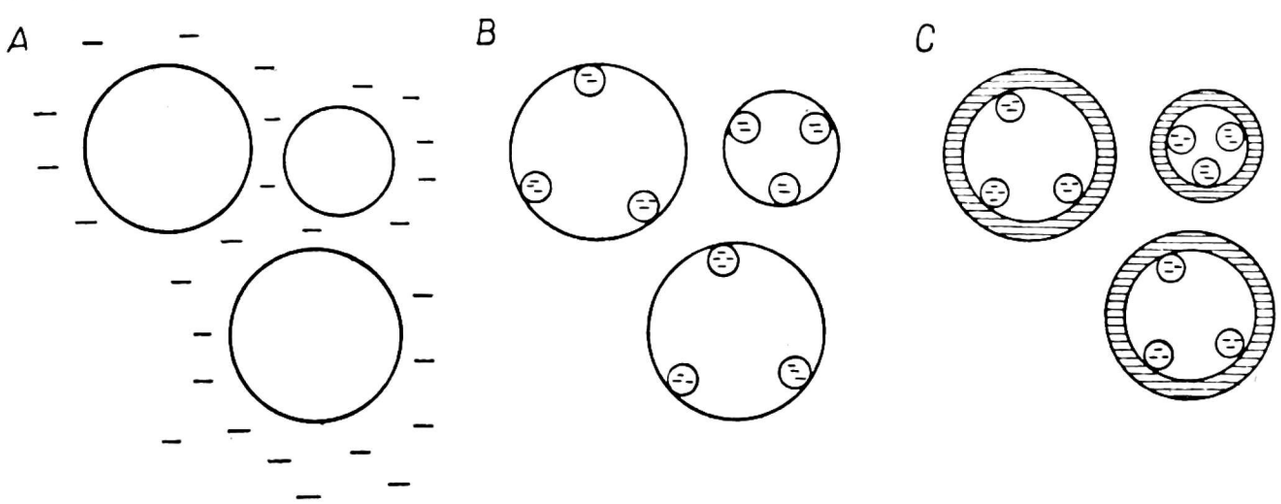

Fig. 1. Schematic representation of "inoculation" of isolated protoplasts by virus. *A* — incubation of virus, *B* — uptake of virus, *C* — cell wall regeneration following uptake of virus.

The potential of such isolated protoplasts in the elucidation of the early stages of infection of plant cells by viruses is currently arousing considerable interest. Takebe *et al.* [8] have been able to isolate protoplasts from tobacco leaves using both pectinase (to separate the cells) and cellulase (to digest away the cell wall of the isolated cells) and it is likely that they have a comparable potential to these isolated fruit protoplasts for synchronous infection studies with plant viruses.

Initially it was considered that the most direct and unambiguous evidence for any virus multiplication in this "inoculated" regenerated protoplast system would be obtained from electron microscopic observations on thin sections of suitably fixed and embedded material.

MATERIALS AND METHODS

The detailed experimental approach to the culture of these "inoculated" regenerated protoplasts has recently bee fully described [2]. Rather than present experimental detail of purification procedures it will be most useful to emphasise those aspects of the experimental approach which are essential for reproducible infection to be obtained.

In preliminary studies it became clear that adequate removal of virus inhibitory materials from the pectinase used to isolate the protoplasts from the licule tissue of mature virus free green tomato fruit was essential. This is most readily achieved by chromatography of pectinase (Sigma Chemical Co. Ltd.) on Sephadex G 100. Ribonucleases are a major source of difficulty in obtaining reproducible infection in this system and these are readily removed from pectinase using Sephadex G 100.

RESULTS

When these isolated protoplasts are incubated with 1% tobacco mosaic virus in 20% sucrose for 6 hr, and the protoplasts are fixed in glutaraldehyde and then post fixed in OsO_4 and sections of the protoplasts embedded in butyl methacrylate styrene, virus is detected in vesicles in the cytoplasm. Only a few vesicles contain virus particles (Fig. 2). In Fig. 3 which shows one of these vesicles at higher magnification several particles of virus are clearly visible. There is some suggestion that these particles of tobacco mosaic virus are being degraded within these vesicles. These particles are somewhat thinner than is usual with this particular virus stained during dehydration with uranyl acetate [3]. Moreover, after about 30 hr of culture of regenerated protoplasts following pinocytic uptake of virus no virus is visible within vesicles in the cytoplasm. After 120 hr of culture a marked multiplication of virus can be detected. Aggregates of virus are visible at this stage in the cytoplasm of these regenerated protoplasts. A typical region of the cytoplasm with virus clearly visible is shown in Fig. 4.

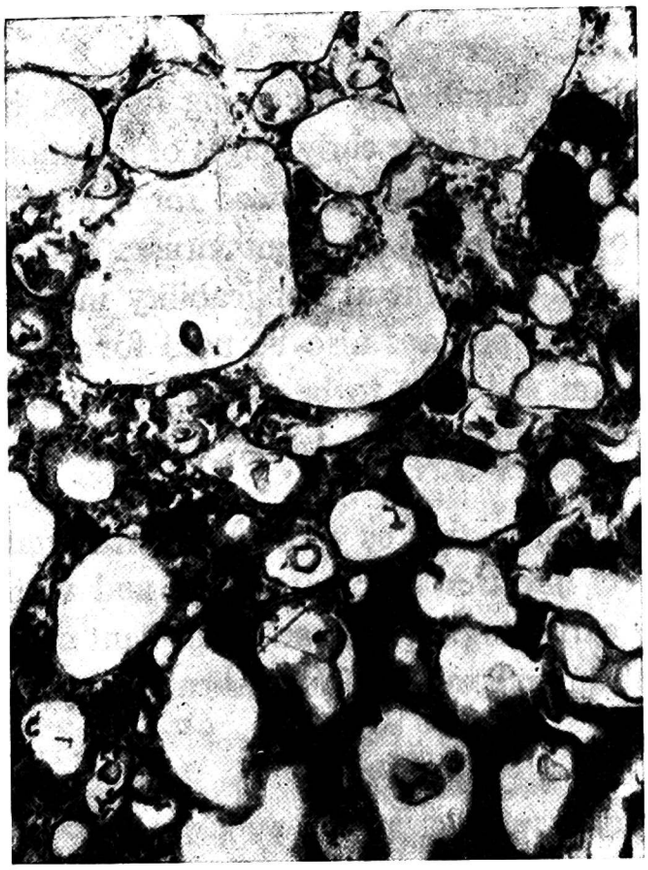

Fig. 2. Low magnification of region of cytoplasm of isolated protoplast after 6 hr incubation with tobacco mosaic virus. Note virus in vesicle (in rectangle).

Fig. 3. Higher magnification of region of pinocytic vesicle in Fig. 2. Note virus particles V within the vesicle.

Fig. 4. Aggregates of tobacco mosaic virus in the cytoplasm of infected regenerated protoplast after 120 hr culture. Virus (V).

DISCUSSION

The demonstration that these protoplasts are capable of becoming infected is now beginning to provide us with an exciting insight into the early stages of infection of cells by tobacco mosaic virus. Siegel [7] has emphasised the need for a model system in which cells can become synchronously infected by plant viruses and it would seem likely that this protoplast system has a general applicability in this respect. The isolated leaf protoplast system could perhaps also be used for comparable infection studies but particular attention will have to be paid to the purification of the pectinase and cellulase enzymes employed to isolate these protoplasts [8].

It is clear that this experimental approach is providing us with an experimental system in which it is possible to obtain direct infection by plant viruses and with which we can begin to investigate more fully early stages of infection of plant cells by viruses and thereby bring to plant virology the more exact and sophisticated approaches adopted in animal virology.

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