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Replication of *Oryctes* Baculovirus in Cell Culture: Viral Morphogenesis, Infectivity and Protein Synthesis

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SUMMARY

The sequence of events in the replication of *Oryctes* baculovirus in DSIR-HA-1179 cells began with the uptake of virus particles by pinocytosis at the plasma membrane. Seven h after infection, enveloped virus particles were observed inside a cleared area in the nucleus. Virus was released from cells by budding through the plasma membrane where a second unit membrane was acquired. The infectivity of virus particles with a single envelope purified from within cells was not significantly different from that of virus which had acquired the second envelope by budding. Twenty-seven structural virus proteins were identified by gradient polyacrylamide gel electrophoresis. The synthesis of eight of the major structural proteins was detected by pulse labelling infected cells with L-[³⁵S]methionine. No late protein synthesis, as observed in subgroup A baculoviruses, was detected in this subgroup C baculovirus.

INTRODUCTION

Oryctes baculovirus was discovered by Huger (1966) in diseased rhinoceros beetle (*Oryctes rhinoceros* L.) larvae in Malaysia. Since its first release as a microbial control agent in Samoa it has been spectacularly successful in controlling the coconut palm rhinoceros beetle throughout the tropics (Marschall, 1970; Zelazny, 1976; Bedford, 1980). *Oryctes* baculovirus is also the type species of subgroup C baculoviruses within the family Baculoviridae. Unlike subgroups A and B of this family, viruses of subgroup C are not found occluded within a crystalline protein inclusion body (Matthews, 1982). Subgroup C baculoviruses have been found in a wide range of arthropod hosts including most insect orders, mites and crustacea (Crawford & Granados, 1982). With the exception of *Oryctes* baculovirus and a virus found to infect the IMC-HZ-1 cell line persistently (Granados *et al.*, 1978), no other subgroup C baculoviruses have been shown to replicate in cell culture.

The first report of *Oryctes* baculovirus replication in cell culture was in a primary cell culture derived from *O. rhinoceros* (Quiot *et al.*, 1973). A further report of *Oryctes* baculovirus replication in *Spodoptera frugiperda* (J. E. Smith) and *Aedes albopictus* (Skuse) cell lines (Kelly, 1976) has been disputed (Crawford, 1981). Our paper provides details of *Oryctes* baculovirus replication in the cell line DSIR-HA-1179 (HA cells) derived from *Heteronychus arator* (F.) (Coleoptera: Scarabaeidae) (Crawford, 1982). Viral morphogenesis, the infectivity of virus particles and the synthesis of viral proteins are examined.

METHODS

Virus and cells. The *Oryctes* baculovirus used in these experiments, denoted as strain PV505, is the strain used for the initial characterization of the virus nucleocapsid (Payne *et al.*, 1977). One frozen infected adult beetle was kindly provided by Dr C. C. Payne (Glasshouse Crops Research Institute, Littlehampton, U.K.). The midgut was dissected out, homogenized and filtered through a 0.22 µm membrane. The aseptic filtrate was used as an inoculum for HA cells. The virus obtained was cloned by three serial transfers at limiting dilution to give a virus stock with a probability of 600:1 that it came from a single virus particle. Virus particles and nucleocapsids were purified as previously described (Payne *et al.*, 1977).

The HA cell line, used for all virus replication and morphogenesis studies, was derived from the scarab beetle *H. arator* (Crawford, 1982). For these studies, the cells were grown in Schneider's medium (Gibco) containing 10% foetal calf serum and 50 µg/ml gentamicin at 27 °C. Viruses were titrated using a tissue culture infectivity endpoint dilution assay ($TCID_{50}$) performed in 60-well microtest II plates (Falcon) as previously described (Crawford & Granados, 1982). The $TCID_{50}$ titre was calculated by the statistical method of Reed & Muench (1938). The titre was expressed in infectious units (IU) by assuming that the probability of infection of a single well was according to a Poisson distribution where $IU = 0.69 \times TCID_{50}$ titre.

Electron microscopy. Methods of sample fixation, embedding and sectioning have been described previously (Crawford, 1981); however, for these studies cells were fixed and embedded *in situ*. The cells, grown as monolayers in 25 cm² plastic flasks (Falcon), were fixed, embedded and sectioned still attached to the surface of the flask.

Preparation of budded and cell-associated virus. Culture fluid (10 ml) from an infected HA cell culture at 72 h post-infection containing 2×10^6 cells was centrifuged (1000 g, 10 min) to pellet any floating cells and the supernatant was retained. The cell pellet was returned to the flask containing the majority of cells still attached. Ten ml of fresh medium was added and the cells were disrupted by sonication for 30 s at full power with a Kontes sonicator (Vineland, N.J., U.S.A.) to release cell-associated virus. Budded virus was sonicated in an identical fashion. Microscopic examination of the flask showed that all cells and nuclei were disrupted. No attempt was made to purify further either the cell-associated (CA) or budded (Bud) viruses before they were assayed for infectivity as any purification step may have affected their viability. Sonication of the Bud virus preparation did not significantly alter its infectivity (data not shown).

Physical/infectious particle ratio determination. To determine the physical concentration of virus particles the viral DNA content of the CA or Bud virus sample was assayed using DNA-DNA dot-blot hybridization (Thomas, 1980). CA and Bud virus samples were pelleted (100000 g, 1 h). The pellets were resuspended and incubated for 2 h at 37 °C in 300 µl 0.1 M-Tris-HCl pH 8.0, 10 mM-EDTA, 0.1% SDS and 0.5% Pronase. The digested pellets were extracted three times with chloroform : phenol saturated with 0.1 M-Tris buffer (pH 7.6) : isopropanol (24 : 24 : 1). The final aqueous phases and a sample of purified standard *Oryctes* viral DNA were melted (100 °C, 5 min), and a twofold dilution series was made. Five µl of each dilution was spotted onto a nitrocellulose membrane (Millipore 0.45 µm). The filter was dried and baked (80 °C, 2 h). The viral DNA bound to the filter was then detected by hybridization to labelled DNA (Thomas, 1980). The labelled DNA probe was prepared by nick translation (Rigby *et al.*, 1977) to a specific activity of 7×10^6 c.p.m./µg using L-[³⁵S]dATP (Amersham). The amount of virus-specific DNA in the CA and Bud DNA virus preparations was estimated by comparison with the amount of probe bound to purified standard *Oryctes* baculovirus DNA.

Radiolabelling of virus and cells. Cells were grown as monolayers in 12-well plastic plates (Linbro 76-053-05). Each 4.5 cm² well contained 8×10^4 cells. Incorporation of L-[³⁵S]methionine (800 µCi/mmol, Amersham) was carried out in modified Schneider's medium (Gibco) without L-methionine or foetal calf serum. Cells were infected at a multiplicity of 100 i.u./cell. After 30 min the inoculum was removed, the monolayer was washed three times with modified Schneider's medium and the cells incubated at 27 °C in modified medium until pulse-labelled with L-[³⁵S]methionine at a concentration of 50 µCi/ml in modified medium.

Gel electrophoresis. The pulse-labelled cells were scraped from the surface of the flask and centrifuged at 1000 g for 5 min. The supernatant was discarded and the cell pellet lysed in SDS-PAGE sample buffer (Laemmli, 1970). Samples were electrophoresed on a 5 to 15% gradient polyacrylamide gel with a 3% stacking gel, using the discontinuous buffer system of Laemmli (1970). The gel was impregnated with scintillant, dried, and autoradiographed with preflashed X-ray film (Bonner & Laskey, 1974). Molecular weight determinations were made by reference to ¹⁴C-methylated myosin, phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase and lysozyme (Amersham), with molecular weights of 200000, 92500, 69000, 46000, 30000 and 14300 respectively.

Unlabelled proteins from purified virus and nucleocapsids were detected in gels using silver staining (Eschenbruch & Bürk, 1982).

RESULTS

Viral cytopathic effect and morphogenesis

The first sign of c.p.e. caused by *Oryctes* baculovirus in HA cells was that the fibroblast-like cells became spherical. Complete cytolysis, in which many of the cells fragmented, occurred within 3 to 4 days (Fig. 1a). This c.p.e. is the basis of the $TCID_{50}$ assay used to measure viral infectivity (Crawford & Granados, 1982). Repeated attempts to obtain virus plaques on HA cell monolayers have failed.

Despite examining between 500 and 1000 infected cells at 0.5 to 4 h and infecting cells at a very high multiplicity (approx. 100 i.u./cell), we found very few intracellular virus particles.

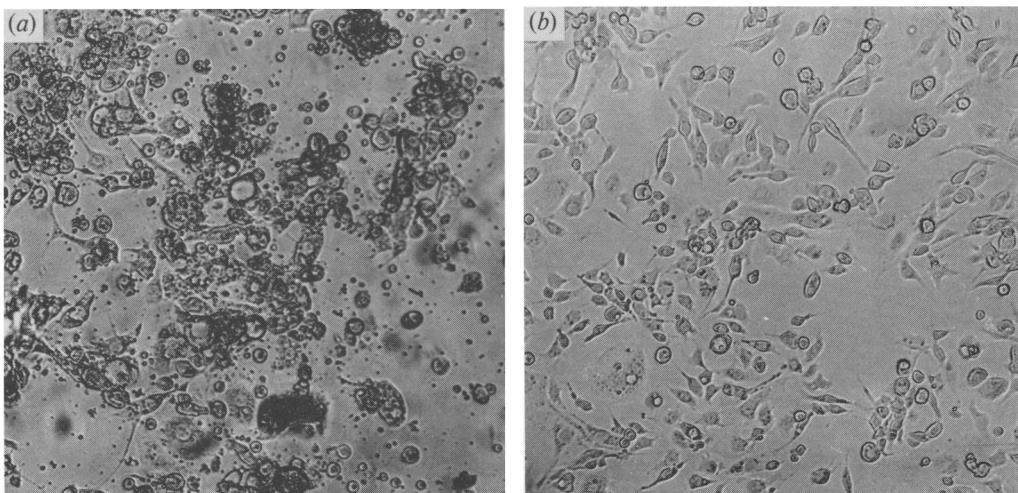


Fig. 1. (a) Typical *Oryctes* baculovirus c.p.e. in HA cells. (b) Healthy uninfected HA cells.

Virus particles were observed within vesicles near the plasma membrane (Fig. 2a). This suggests that they enter the cell by pinocytosis. The only unenveloped nucleocapsid observed in this study was seen in the cytoplasm during the first 4 h of infection (Fig. 2b).

The earliest sign of replication occurred in the nucleus at approximately 7 h where cleared, chromatin-free areas were observed (Fig. 2c, arrowed). A few of the cleared areas observed at 7 h post-infection contained several enveloped nucleocapsids. By 12 h, however, replication was at its height, with large numbers of enveloped virus particles observed within the cleared area of the nucleus (Fig. 3a). Unenveloped nucleocapsids, as observed during morphogenesis of groups A and B baculoviruses, were never observed. However, empty nucleocapsid shells surrounded by a unit membrane were observed in the nucleus (Fig. 3a, arrowed). We therefore suggest that an envelope and nucleocapsid shell form first. The shell is then filled with an electron-dense core. Enveloped nucleocapsids were also observed arranged along the nuclear membrane (Fig. 3b). As early as 12 h post-infection, virus was observed budding from the plasma membrane where it acquired a second unit membrane. From 12 to 36 h post-infection the cells showed little change. Virus was observed in both nucleus and cytoplasm and at both nuclear and plasma membranes. The cell in Fig. 3(c) (16 h after infection) shows virus at later stages of morphogenesis. From 16 h envelope material accumulated (Fig. 3c, open arrows) within the cleared area of the nucleus. The greatest incidence of virus budding was observed at 16 h post-infection (Fig. 4a, b). It continued to occur until at least 36 h after infection. A second unit membrane was acquired as the virus budded from the plasma membrane (Fig. 4a, white arrow). Some extracellular virus rods cut transversely showed an unusual bulge in one side of the envelopes (Fig. 4a, b, closed black arrows). It is possible that this accommodates the tail-like structure Payne *et al.* (1977) first observed attached to the nucleocapsid.

Comparison of infectivity between cell-associated and budded virus

Our estimates of virus production from HA cells infected with *Oryctes* baculovirus showed 380 i.u. of Bud virus was produced per cell and that a further 300 i.u./cell of CA virus remained within the infected tissue. The *Oryctes* baculovirus DNA content of 5×10^5 CA i.u. and 6.5×10^5 Bud i.u. was estimated to be 2 ng using the dot-blot assay (Fig. 5). The physical to infectious particle ratios are therefore 2.5×10^8 i.u./ μg CA DNA (24 genome copies/i.u.) and 3.2×10^8 i.u./ μg Bud DNA (18 genome copies/i.u.) respectively. The difference between the ratios is not significant ($P < 0.05$).

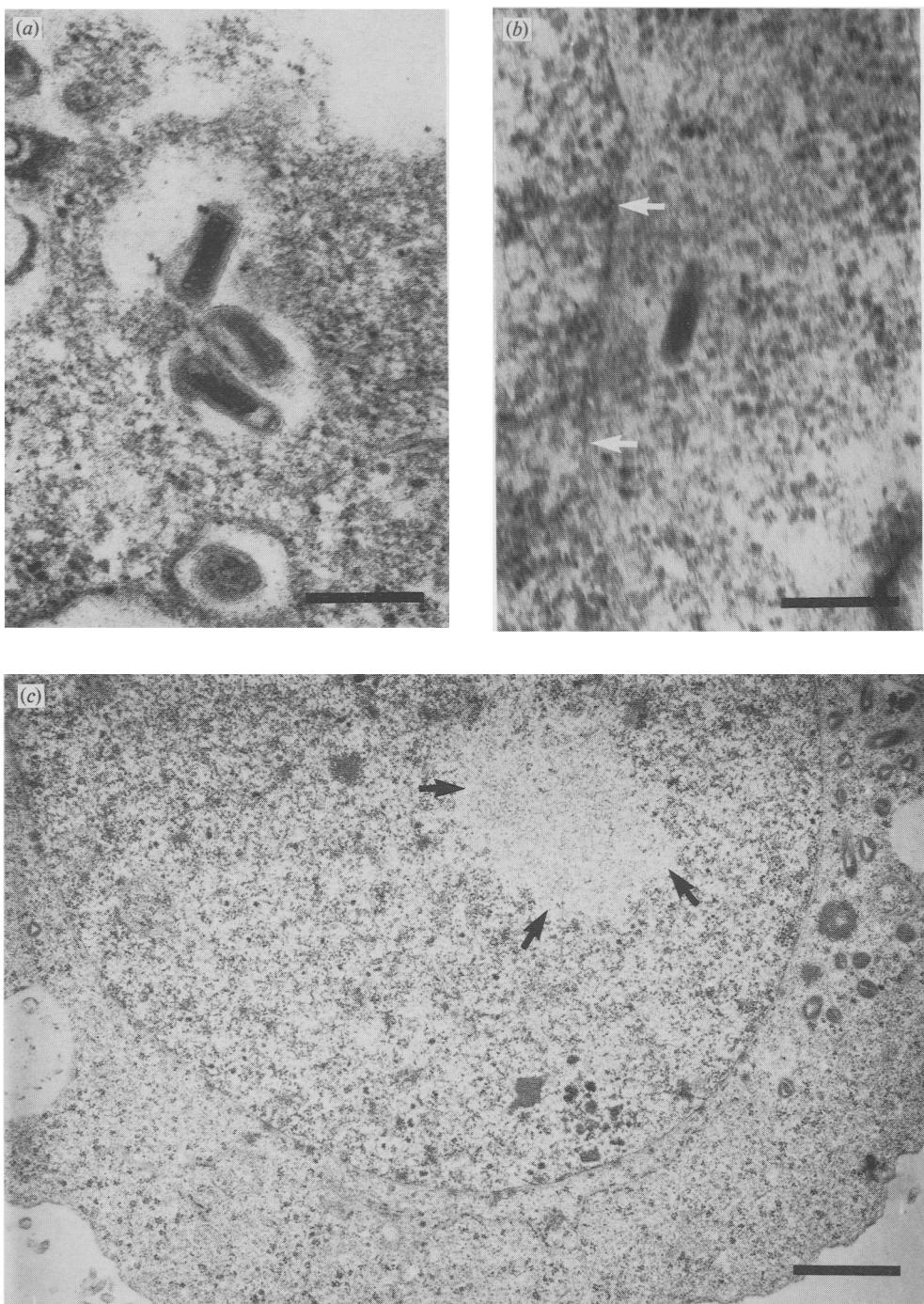


Fig. 2. (a) Virus particles within vesicles in the cytoplasm at 1 h post-infection. Bar marker represents 200 nm. (b) Single nucleocapsid observed in the cytoplasm. White arrows indicate nuclear membrane. Bar marker represents 200 nm. (c) Earliest sign of virus replication. An electron-lucent area of the nucleus at 7 h post-infection is arrowed. Bar marker represents 1 μ m.

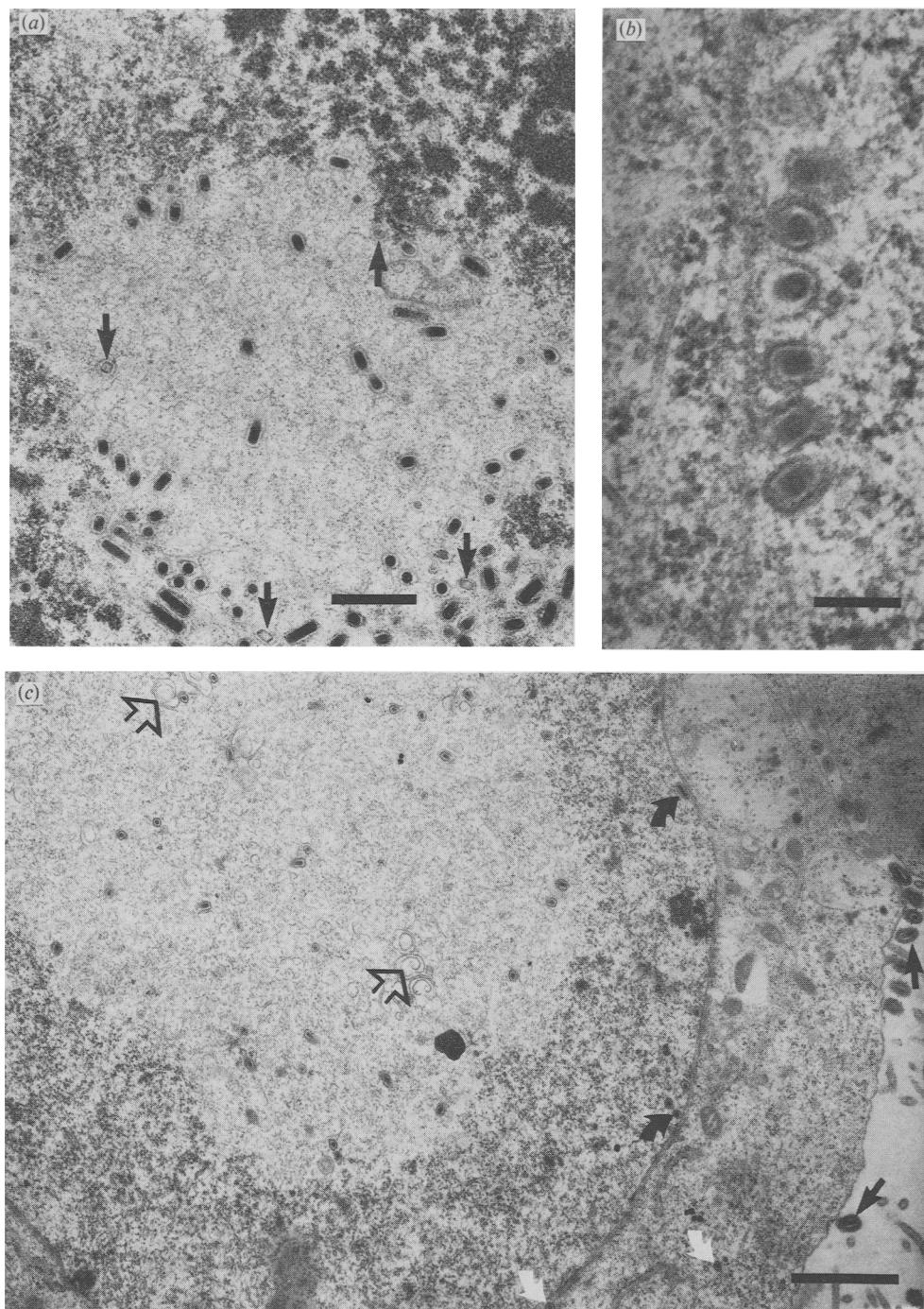


Fig. 3. (a) Formation of virus particles within the electron-lucent area of the nucleus. Apparently empty nucleocapsids are arrowed. Bar marker represents 400 nm. (b) A rarely found line of virus particles arranged along the inner nuclear membrane at 16 h post-infection. Bar marker represents 150 nm. (c) Infected cell at 16 h post-infection. Note the build-up of envelope material in the cleared area of the nucleus (open arrows), the virus particles at the nuclear membrane (black curved arrows), the virus in the cytoplasm (white arrows) and the extracellular budded virus with two unit membranes (straight black arrows). Bar marker represents 1 μ m.

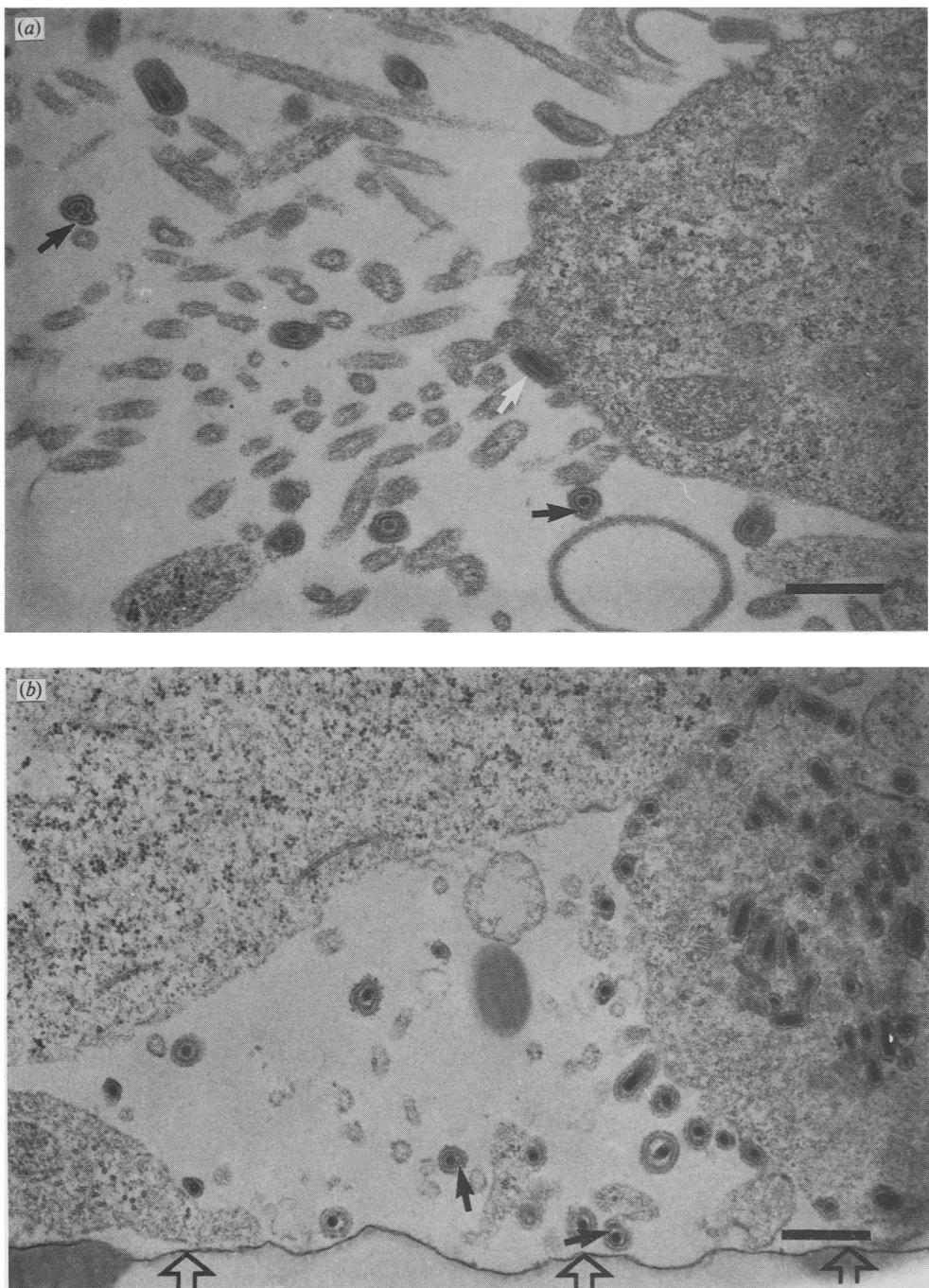


Fig. 4. Two sections of infected cells at 16 h post-infection with virus budding from the plasma membrane. The second membrane is acquired as the virus buds through the plasma membrane. A virus in the process of budding (*a*) shows two membranes on its exterior surface but only one on its interior surface. Extracellular virus particles cut transversely show a bulge to one side (black arrows in *a* and *b*), which may hold the nucleocapsid tails first identified by Payne *et al.* (1977). The *in situ* nature of the sectioning procedure is shown in (*b*), where the surface of the flask, transversely cut, is clearly visible (open arrows). Bar markers represent 400 nm.

Doubling dilutions ↑

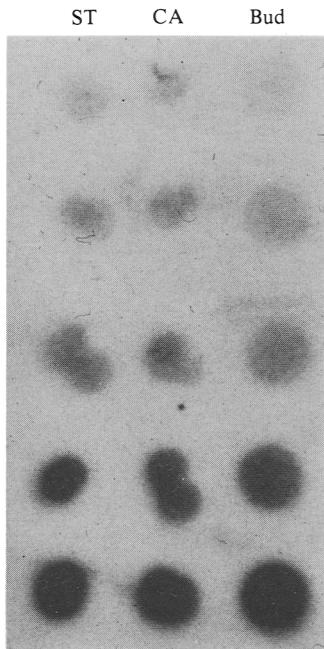


Fig. 5

Fig. 5. Dot-blot assay for *Oryctes* baculovirus DNA. The largest amount of standard (ST) DNA in a dot contained 2 ng of *Oryctes* baculovirus DNA, the lowest CA DNA spot contained DNA from 5×10^5 i.u. and the lowest Bud DNA spot contained DNA from 6.5×10^5 i.u.

Fig. 6. Proteins of *Oryctes* baculovirus separated by 5 to 15% gradient PAGE and stained with silver. (a) Proteins of purified whole virus; (b) proteins of virus nucleocapsids prepared by treatment of virus with 1% NP40 at 37 °C for 1 h. Numbers indicate protein mol. wt. $\times 10^{-3}$.

Virus structural proteins and protein synthesis

A total of 27 proteins were detected in silver-stained polyacrylamide gels (Fig. 6). When virus was treated with the non-ionic detergent NP40, which has been shown previously to remove the virus envelope (Payne *et al.*, 1977), 14 protein bands disappeared or were markedly reduced. It is therefore probable that these 14 proteins are contained in the envelope whereas the remaining proteins are associated with the nucleocapsid.

Pulse labelling infected cells with L-[³⁵S]methionine at 2 h intervals during infection enabled the synthesis of only eight viral polypeptides to be observed. *Oryctes* baculovirus, like the

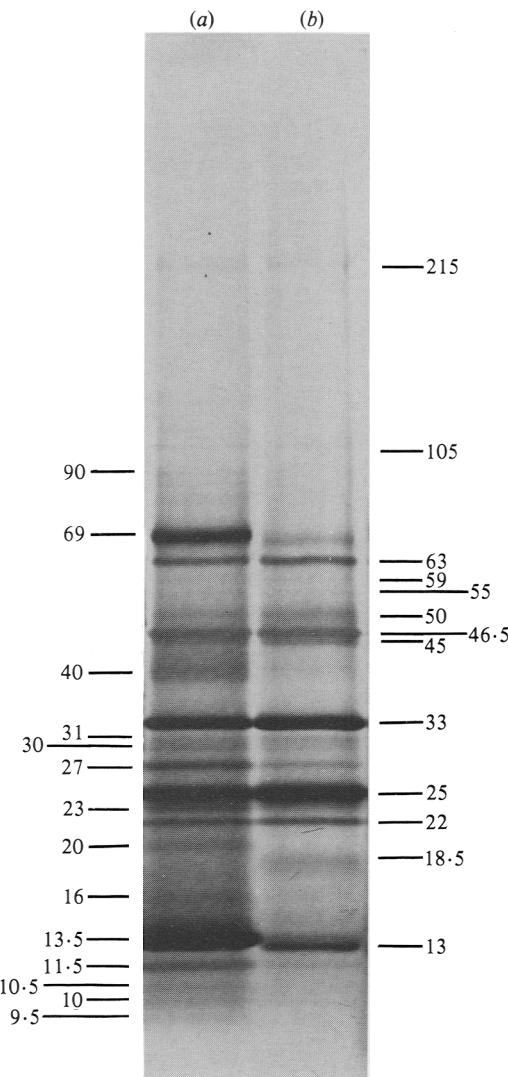


Fig. 6

Table 1. *Viral protein synthesis in Oryctes baculovirus-infected HA cells*

Protein (mol. wt. $\times 10^{-3}$)*	Observed duration of synthesis (h post-infection)†	
	From	To
p46.5	4	20
p40	6	18
p27	6	30
p25	6	32
p22	6	28
p13	6	36
p11.5	10	18
p10	4	18

* Molecular weight was determined by co-migration with ^{14}C -methylated protein standards (Amersham).

† Duration of synthesis was assessed from autoradiographs as in Fig. 7.

baculoviruses of subgroups A and B (Carstens *et al.*, 1979; Dobos & Cochran, 1980; Wood, 1980), did not depress host protein synthesis a great deal even when infected at high multiplicity (m.o.i. 100). The most abundant protein, p13 (arrowed), was synthesized up to 36 h after infection. The other proteins, however, ceased to be detected before this. A summary of these results is presented in Table 1. All proteins except p11.5 were first detected between 4 and 8 h after infection (Fig. 7). All the synthesized proteins detected are probably structural as their molecular weights all correspond to structural proteins detected on silver-stained gels (see Fig. 6) of purified virus. No protein synthesis analogous to the inclusion body protein synthesis found in subgroup A baculoviruses was observed.

DISCUSSION

Our studies on the morphogenesis of *Oryctes* baculovirus are summarized in Fig. 8. The presence of virus in vesicles near the plasma membrane indicates virus entry by pinocytosis. We conclude that following entry the virus particles are rapidly degraded, as only one virus particle was observed in extensive searches of the cytoplasm and nuclear membranes between 0.5 and 4 h post-infection.

Oryctes baculovirus has been observed only as enveloped nucleocapsids in thin section (Huger, 1966; Payne, 1974), whereas subgroups A and B baculoviruses form as naked nucleocapsids and acquire envelopes either during budding from the plasma membrane or before occlusion into polyhedra or granules (Summers & Volkman, 1976). Our results showed that *Oryctes* baculovirus was observed as early as 7 h after infection in the nucleus of infected cells and it was always observed surrounded by a unit membrane.

Electron-lucent or 'empty' nucleocapsids were occasionally observed in the nucleus. This has occasionally been observed with other subgroup C baculoviruses (Gouranton, 1972; Kim & Kitajima, 1984). The probable morphogenesis of subgroup C baculoviruses is therefore that the envelope and nucleocapsid shell assemble first, followed by insertion of the electron-dense core containing the viral DNA. A subgroup C baculovirus from the parasitic wasp *Mesoleius tenthredinis* is the only other member of this group of viruses observed budding from the plasma membrane (Stoltz, 1980). It also acquired a second unit membrane at the cell surface.

The productivity of *Oryctes* baculovirus infections (380 i.u. budded/cell) was higher than that of any other reported baculovirus-infected cells. This was probably because HA cells are large (Crawford, 1982), occupying approximately three times the substrate surface area of TN368 cells and ten times the area of *S. frugiperda* cells. Our infectivity assay is approximately four times more sensitive than that reported for *Autographa californica* nuclear polyhedrosis virus in TN368 cells (Volkman *et al.*, 1976).

Our protein studies identified 15 proteins in addition to those previously reported to be associated with the virus particle (Payne *et al.*, 1977). The sequence of synthesis of eight of the structural proteins was defined. The time course of viral protein synthesis and the incomplete

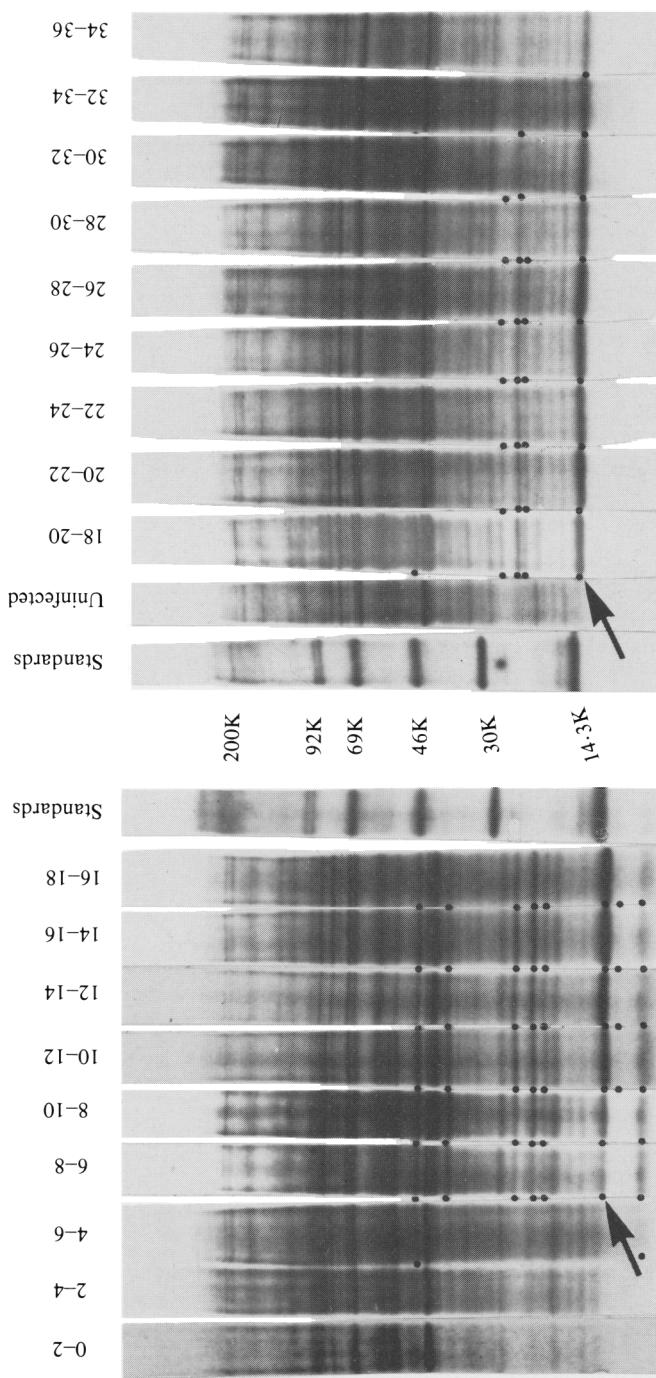


Fig. 7. Autoradiogram of *Ornytes* baculovirus-infected HA cells pulse-labelled with L-[³⁵S]methionine from 0 to 36 h post-infection. The numbers above each lane indicate the timing of each 2 h pulse after infection. The dots show the location of presumptive viral proteins. P13, the major nucleocapsid protein, is arrowed.

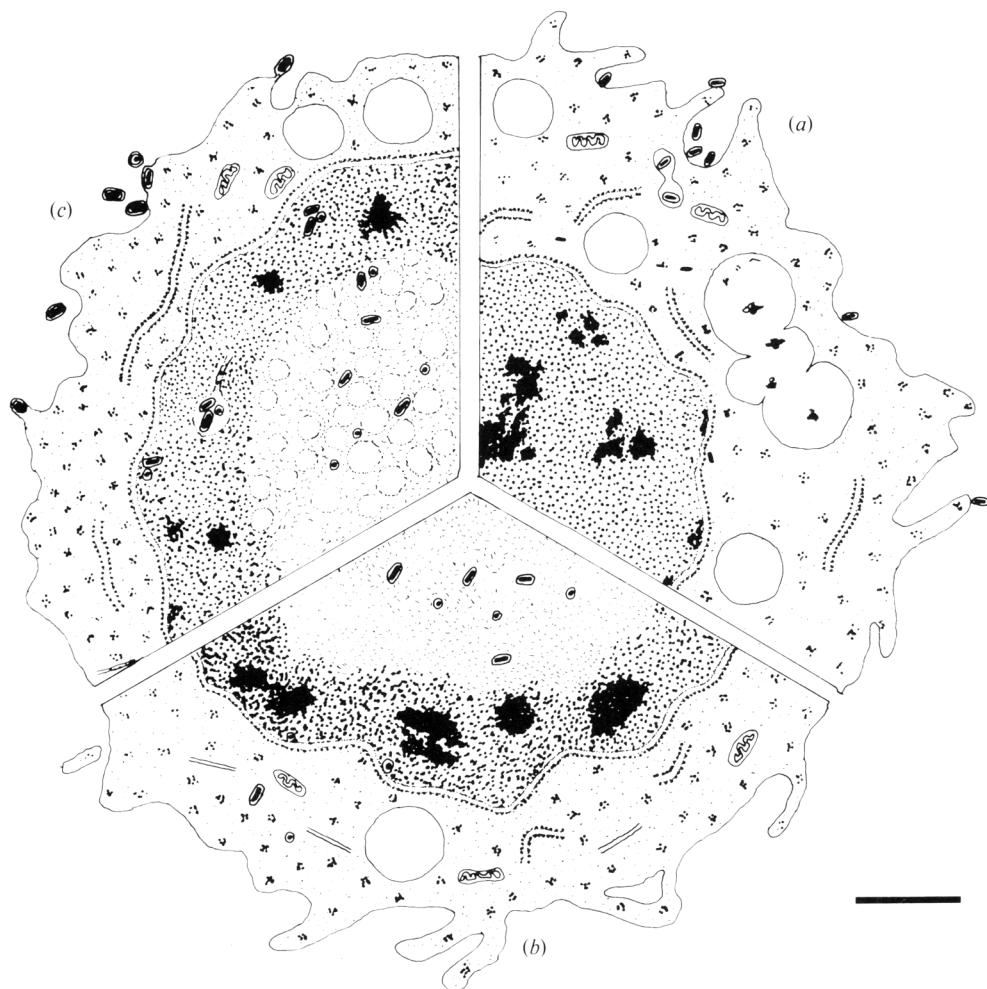


Fig. 8. Summary of morphological changes in HA cells infected with *Oryctes* baculovirus. (a) 1 to 4 h post-infection: virus adsorption to the plasma membrane and uptake in cytoplasmic vesicles. (b) 7 to 12 h post-infection: viral replication in the clear area of the enlarged nucleus. (c) 16 and over h post-infection: virus release by budding from the plasma membrane and the characteristic accumulation of envelope material in the cell nucleus. Bar marker represents 1 μ m.

blocking of host protein synthesis appeared similar to that reported for subgroup A baculoviruses (Wood, 1980). The absence of new proteins synthesized late in the infection and analogous to inclusion body protein was the only major difference observed.

This study has shown three areas of difference between *Oryctes* baculovirus, a subgroup C baculovirus, and the more intensively studied subgroup A baculoviruses. These were the absence of naked nucleocapsids, the acquisition of a second unit membrane by those particles budded from the plasma membrane, and the absence of 'late' protein synthesis. Smith & Summers (1982) have shown that certain baculovirus DNA sequences are conserved among many baculoviruses of subgroups A and B and also with the Hz-1 baculovirus. It will be interesting to see whether these conserved sequences can be found in *Oryctes* baculovirus.

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REFERENCES

- BEDFORD, G. O. (1980). Biology, ecology and control of palm rhinoceros beetles. *Annual Review of Entomology* **25**, 309–339.
- BONNER, W. M. & LASKEY, R. A. (1974). A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. *European Journal of Biochemistry* **46**, 83–88.
- CARSTENS, E. B., TIJIA, S. T. & DOERFLER, W. (1979). Infection of *Spodoptera frugiperda* cells with *Autographa californica* nuclear polyhedrosis virus. *Virology* **99**, 386–398.
- CRAWFORD, A. M. (1981). Attempts to obtain *Oryctes* baculovirus replication in three insect cell cultures. *Virology* **112**, 625–633.
- CRAWFORD, A. M. (1982). A coleopteran cell line derived from *Heteronychus arator* (Coleoptera: Scarabaeidae). *In Vitro* **18**, 813–816.
- CRAWFORD, A. M. & GRANADOS, R. R. (1982). Non-occluded baculoviruses. In *Proceedings of Third International Colloquium on Invertebrate Pathology* (Brighton, U.K.), pp. 154–159.
- DOBOS, P. & COCHRAN, M. A. (1980). Proteins in cells infected by *Autographa californica* nuclear polyhedrosis virus (Ac-NPV): the effect of cytosine arabinoside. *Virology* **103**, 446–464.
- ESCHENBRUCH, M. & BÜRK, R. R. (1982). Experimentally improved reliability of ultrasensitive silver staining of protein in polyacrylamide gels. *Analytical Biochemistry* **125**, 96–99.
- GORANTONI, J. (1972). Development of an intranuclear nonoccluded rod-shaped virus in some midgut cells of an adult insect, *Gyrinus natator* L. (Coleoptera). *Journal of Ultrastructure Research* **39**, 281–294.
- GRANADOS, R. R., NGUYEN, T. & CATO, B. (1978). An insect cell line persistently infected with a baculovirus like particle. *Intervirology* **10**, 309–317.
- HUGER, A. M. (1966). A virus disease of the Indian rhinoceros beetle *Oryctes rhinoceros* (Linnaeus), caused by a new type of insect virus, *Rhabdionvirus oryctes* gen. n., sp. n. *Journal of Invertebrate Pathology* **8**, 38–51.
- KELLY, D. C. (1976). 'Oryctes' virus replication: electron microscopic observations on infected moth and mosquito cells. *Virology* **69**, 596–606.
- KIM, K. S. & KITAJIMA, E. W. (1984). Non-occluded baculovirus- and filamentous virus-like particles in the spotted cucumber beetle, *Diabrotica undecimpunctata* (Coleoptera: Chrysomelid). *Journal of Invertebrate Pathology* **43**, 234–241.
- LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, London* **227**, 680–685.
- MARSCHALL, K. J. (1970). Introduction of a new virus disease of the coconut rhinoceros beetle in Western Samoa. *Nature, London* **225**, 288.
- MATTHEWS, R. E. F. (1982). Classification and nomenclature of viruses. *Intervirology* **17**, 52–54.
- PAYNE, C. C. (1974). The isolation and characterization of a virus from *Oryctes rhinoceros*. *Journal of General Virology* **25**, 105–116.
- PAYNE, C. C., COMPSON, D. & DE LOOZE, S. M. (1977). Properties of the nucleocapsids of a virus isolated from *Oryctes rhinoceros*. *Virology* **77**, 269–280.
- QUIOT, J., MONSARRAT, P., MEYNADIER, G., CROIZIER, G. & VAGO, C. (1973). Infection des cultures cellulaires de coléoptères par le 'virus Oryctes'. *Comptes rendus hebdomadaires des séances de l'Académie des sciences, série D* **276**, 3229–3231.
- REED, L. J. & MUENCH, H. (1938). A simple method of estimating fifty percent endpoints. *American Journal of Hygiene* **27**, 493–497.
- RIGBY, P. W. J., DIECKMANN, M., RHODES, C. & BERG, P. (1977). Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *Journal of Molecular Biology* **113**, 237–251.
- SMITH, G. E. & SUMMERS, M. D. (1982). DNA homology among subgroup A, B, and C baculoviruses. *Virology* **123**, 393–406.
- STOLTZ, D. B. (1980). A putative baculovirus in the ichneumonid parasitoid, *Mesoleius tenthredinis*. *Canadian Journal of Microbiology* **27**, 116–122.
- SUMMERS, M. D. & VOLKMAN, L. E. (1976). Comparison of biophysical and morphological properties of occluded and extracellular nonoccluded baculovirions from *in vivo* and *in vitro* host systems. *Journal of Virology* **17**, 962–972.
- THOMAS, P. S. (1980). Hybridisation of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proceedings of the National Academy of Sciences, U.S.A.* **77**, 5201–5205.
- VOLKMAN, L. E., SUMMERS, M. D. & HSIEH, C. H. (1976). Occluded and non-occluded nuclear polyhedrosis virus grown in *Trichoplusia ni*: comparative neutralization, comparative infectivity, and *in vitro* growth studies. *Journal of Virology* **19**, 820–832.
- WOOD, H. A. (1980). *Autographa californica* nuclear polyhedrosis virus-induced protein in tissue culture. *Virology* **102**, 21–27.
- ZELAZNY, B. (1976). Transmission of a baculovirus in populations of *Oryctes rhinoceros*. *Journal of Invertebrate Pathology* **27**, 221–227.

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