Ultrastructural Study of Virus-like Particles in Chinese Hamster Lung Cells

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SUMMARY

Virus-like particles were found in the E36 cells. One type, associated with centrioles, consisted of two concentric shells with a diam. of 50 to 60 nm. Some of these particles were seen budding through the plasma membrane giving rise to a free immature particle showing two concentric shells and an outer envelope. Concentration of the inner shell into a nucleoid results in a mature particle characterized by a nucleoid, mainly eccentrically located, surrounded by an intermediate layer and wrapped in an envelope. The diameter of the mature and immature particles was 75 to 85 nm. The morphogenesis of this virus-like particle resembles that of the oncoviruses.

The following virus particles resembling oncoviruses have been described in hamster cells: (1) C-type particles: in transplantable tumours (Stenback et al. 1966), in tumours induced by MCA or DMBA and by SV40 and polyoma virus (Freeman et al. 1974), and those isolated from hamster tumours originally induced by murine sarcoma virus (Kelloff et al. 1973); (2) R particles of about 100 nm located in the endoplasmic reticulum, characterized by an outer membrane and a spherical centrally located electron-dense nucleoid with radial ‘spoke-like’ rays which may be in contact with the outer membrane. These particles were first found by Bernhard & Tournier (1964) in the BHK21 cell line and later in several transplantable melanomas (Epstein et al. 1968; Takahashi & Mishima, 1969); and (3) Wheatley (1974) has described virus-like particles closely associated with the centrioles in CHO-K1 and CHO-10 sublines derived from Chinese hamster ovary cells. These particles were spherical and measure 55 to 64 nm with a central core of about 35 to 40 nm which sometimes appears hollow and sometimes dense.

In this brief report we describe virus-like particles in the E36 cell line derived from Chinese hamster lung cells. Two kinds of particle were found, probably belonging to the same virus: virus-like particles in the cytoplasm associated with the centrioles, similar to the virus particles described by Wheatley (1974) in CHO cell line; and extracellular virus-like particles resembling the immature and mature forms of the oncoviruses but different from the classical B-type and C-type particles.

The E36 cell line used in this study was derived from a subclone of V79 (Ford & Yerganian, 1958) designated A3. V79 was derived from the lung of a male Chinese hamster.

The cells were grown in Dulbecco’s modification of Eagle’s medium (Gibco) containing 10 % foetal calf serum (Flow). Cells were fixed in 2.5 % glutaraldehyde in 0.1 M-cacodylate buffer, postfixed in 1 % osmium tetroxide in the same buffer. After fixation the cells were scraped off the dishes with a rubber policeman and centrifuged. The pellet was dehydrated and embedded in a mixture of Epon and Araldite. Thin sections were stained with uranyl acetate and lead hydroxide. The preparations were examined with a Philips electron microscope EM-300 or EM-301.

Virus-like particles similar to those described by Wheatley (1974) were found associated
with centrioles (Fig. 1a). These particles were abundant and characterized by two concentric shells (Fig. 1a, b), the inner one being more electron-dense. The diam. of the outer shell was 50 to 65 nm, while the diam. of the core was 25 to 35 nm. Sometimes these particles were surrounded by amorphous material and some of these particles had inside the core a small electron-dense granule (Fig. 1b).

A few of these intracytoplasmic particles were seen near the cell membrane (Fig. 2a, c) and in a budding stage through the plasma membrane (Fig. 2b). After budding off, these particles gave rise to the free immature forms (Fig. 2d), consisting of two concentric shells similar to the intracytoplasmic particle and an outer envelope. After condensation of the inner shell of the immature particle into a nucleoid the mature form was produced. The mature particle (Fig. 2c, f) is characterized by an electron-dense nucleoid, mainly eccentrically located, separated from an intermediate layer by a translucent space and wrapped in an envelope. Very often some particles were found with a half-condensed nucleoid (Fig. 2e), whose morphology was between that of the mature and immature form.

The outer diameter of the mature and immature particles was 75 to 85 nm. In contrast to the intracytoplasmic particles, budding, free immature and mature particles were scarce.

From this morphological study it seems that the intracytoplasmic, budding, immature and mature particles described here are different stages in the morphogenesis of the same virus, because in the budding and immature particles the intracytoplasmic particle can be recognized. However, for definitive confirmation it is necessary to demonstrate an immunological cross reaction between these types of particles.

The morphogenesis of this virus resembles that of the oncoviruses and more closely that of B-type, Mason Pfizer monkey virus and guinea pig leukaemia virus than that of mammalian C-type particles (see schematic representation of the morphogenesis of oncoviruses in Calafat & Ressang, 1977), because intracytoplasmic particles are found in the first group and not in the morphogenesis of the C-type particles from mammals. On the other hand these hamster virus-like particles cannot be assigned to one of the known virus genera. The particles are significantly smaller than other oncoviruses; the extracellular enveloped particle is 75 to 85 nm and the intracytoplasmic particle is 50 to 65 nm whereas the
enveloped oncoviruses are $\geq 100$ nm and the intracytoplasmic A particles about 70 nm (Dalton & Haguenau, 1973; Dalton et al. 1975). The close association of the intracytoplasmic particles of the hamster with the pericentriolar material, which has served as marker in the isolation of centrosomes from CHO cells (Gould & Borisy, 1977), has never been found in the intracytoplasmic particles of other oncoviruses.

Wheatley (1974) has described only intracytoplasmic particles in CHO cells. It is possible that this cell line only produces the intracytoplasmic particle or that the other types of particles were so scarce that they were overlooked. In the E36 cells, budding and extracellular particles were scarce compared to the abundance of the intracytoplasmic particles. However, it was very easy to find them in all preparations, mainly in the mature form. Wheatley (1974) suggested that the intracytoplasmic particles found in CHO cells could be an A-type precursor of the C-type virus. This explanation is unlikely because until now, no relationship has been found between intracytoplasmic A-type particles and C-type particles from
mammals. In addition, we were not able to detect any major C-type virus proteins in a heterologous radio immunoassay (RIA) using rabbit anti-feline leukaemia virus serum and purified Rauscher leukaemia virus p30 and gp70. Neither could we detect any major B-type virus proteins in a less broad RIA using rabbit anti-C3H-mammary tumour virus and purified gp52 and p27. These experiments indicate that there is no immunological relationship between this virus and the C-type from mammals and the mouse B-type.

Further investigation of the presence of a 70S RNA and RNA-dependent DNA polymerase is necessary to classify this virus as a member of Retroviridae (Dalton et al. 1975).

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REFERENCES


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