NOTES

Murine Intracisternal A Type Particles Fail to Separate from the Membrane of the Endoplasmic Reticulum

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Analysis of serial sections of murine cells containing intracisternal A particles revealed that over 99% of all A particles remain in a budding configuration. This indicates that these particles fail to detach from the membrane of the endoplasmic reticulum. This observation explains how, despite their intracellular abundance in certain murine tumors, no extracellular A-type particles can be found.

The intracisternal virus-like particles associated with mouse neoplasms were first described as A particles by Bernhard (1). They have since been designated intracisternal A particles in order to distinguish them from intracytoplasmic A particles, which are associated with B-type virus (mammary tumor virus) and from C-type virus (2). Intracisternal A particles, 70 to 100 nm in diameter, bud from the rough endoplasmic reticulum into the cisternal space. Although they can occasionally be found in a variety of normal and embryonic tissues (4, 15), they occur in relatively large numbers in a variety of neoplastic cells, including plasma cell tumors (3) and neuroblastomas (7). In a quantitative analysis of clones of Ehrlich ascites cells, Kodama and Kodama (6) found over 15,000 A-type particles per cell in one clone. Despite this high level of cisternal virus-like particles, extracellular particles are never found. In addition, intracisternal A particles have been described in tumors of the rat, gerbil, guinea pig, sheep, and man (9-13).

The exact nature of these virus-like particles remains largely a mystery. Their occurrence at high levels in tumor cells, often in cells also producing type-C particles, has frequently led to the suggestion that they are precursors to type-C particles or are an alternative expression of the type-C genome. It is now generally felt, although not always (5), that intracisternal A particles are completely unrelated to C-type particles (4, 7). Their association with rough endoplasmic reticulum has also led to the suggestion that

intracisternal A particles are associated with protein synthesis and, in fact, are not virus particles at all (3).

Biochemical properties of isolated intracisternal A particles (8), on the other hand, are more consistent with their being unique entities possessing properties typically associated with viruses. For example, cell fractions containing a high concentration of intracisternal A particles contain a unique DNA polymerase (14), high-molecular-weight RNA (16), and an RNA-dependent DNA polymerase (17).

One of the remarkable structural features of intracisternal A particles is the high percentage of particles which appear to be budding at any given time. It seemed conceivable that, if this observation were interpreted in a three-dimensional way, the actual incidence of buds would be still higher. A spontaneously occurring murine rhabdomyosarcoma which contains large numbers of intracisternal A particles was the source of tumor material and tissue culture samples. Samples were prepared for electron microscopy by fixation in 3% glutaraldehyde and post-fixation in 1% osmium tetroxide. The samples were dehydrated in ethanol and propylene oxide and embedded in epon-araldite, and serial sections were cut from these blocks and stained with uranyl acetate and lead citrate. A block of the initial passage level of this tumor was also available, and A. J. Dalton kindly provided blocks of several murine plasma cell tumors. These blocks were freshly cut and stained as described above. All sections were examined with a Siemens 101 electron microscope at 80 kV.

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Intracisternal A particles exhibit considerable morphological variability. Fig. 1A illustrates two budding particles, showing the two electron-dense layers of approximately equal thickness and density separated by an electron lucent space. These two particles appear to illustrate a developmental sequence which presumably would terminate with release of the particle into the cisternal space. Fig. 1B illustrates how the particles and portions of the cytoplasm may form networks extending into large cisternal spaces. The arrow illustrates a particle connected to two other particles and a cytoplasmic membrane.

Following the observation that a high percentage of particles were budding, serial sec-

tions were analyzed for the percentage of particles that were apparently free in a given section. This was done by projecting either the original negative or a positive lantern slide onto an enlarging easel at a final magnification of approximately 160,000. These same particles were then analyzed in each adjacent section to determine what fraction remained apparently detached. Figures 1C and D illustrate examples of two particles which appear detached in one section and attached in an adjacent section (arrows). Table 1 presents the data obtained from analyzing 1,257 particles in serial sections cut from a variety of tumor and tissue culture samples.

Particles in which the two electron-dense

FIG. 1. Intracisternal A particles observed in a murine rhabdomyosarcoma. (A) Budding particles at different stages of development; (B) a network of particles and endoplasmic reticulum extending into a large cisternal space; (C, D) selected examples of two intracisternal A particles connected in one section and apparently unconnected in an adjacent section (arrows). The bars represent 0.1 μ m.

TABLE 1. Counts of mouse intracisternal A particles from serial sections

Source of serial sections	Total particles*	A particles		
		Connected in middle section	Unconnected in middle section	Unconnected in all sections
Tissue culture, rhabdomyosarcoma passage 4	579	519	58	3
Plasma cell tumor	273	240	29	4
Rhabdomyosarcoma tumor passage 1	404	338	59	7
Totals	1,256	1,097	146	14

* Only clearly defined (both layers apparent) particles were considered.

shells were resolvable were identified in a given section and were then located in both adjacent sections to determine if they appeared to be connected to the cisternal membrane. In these initial sections 87.5% of these particles appeared to be attached either to the cisternal membrane or to another particle. Of the 146 particles which did not appear to be attached in the initial sections, 132 were shown to be actually attached by analyzing the neighboring sections. This is equivalent to an overall level of more than 99% attached particles. Essentially the same percentage was obtained regardless of the source of the cells. Since a few buds could be expected to be exactly perpendicular to the plane of sectioning and observation, and thus would always appear to be detached, it is reasonable to assume that in the systems examined practically all intracisternal A particles are in the form of buds.

This finding may help to explain certain properties of the intracisternal A particle. In particular, it appears to explain why no virus-like particles are found extracellularly. If it is an evolutionary derivative of a true virus, i.e., an infectious particle, then the failure to complete budding may simply represent defectiveness in some stage of assembly or maturation. The presence of buds at different stages may indicate a relatively rapid turnover of these particles.

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