DEMONSTRATION OF ENDOGENOUS INTRACISTERNAL TYPE A PARTICLES IN EARLY MOUSE ZYGOTES

G. G. Miller, I. V. Makarova, and A. A. Yazykov

UDC 578.828.1:578.23].086.3+616-013.7-022:578.828.1

KEY WORDS: endogenous intracisternal type A particles; zygote; embryogenesis; differentiation.

It has recently become evident that many aspects of the problem of the origin and evolution of viruses, virus carcinogenesis, genetics, and differentiation are connected with the discovery of endogenous viruses. Among them, the one which has received least study is the group of type A retroviruses. One of them – intracisternal type A particles (IAP) – is regularly found in early mouse [2, 6], hamster [8], cat [4], and other animal embryos. They were first described in mice in 1969 [5] at the 2-8-cell embryo stage, and later in mouse oocytes and parthenogenetically stimulated ova [3, 9, 10]. Expression of IAP in the earliest stages of embryogenesis is an important fact in the understanding of their functions in the normal cell, which have not yet been elucidated. Hitherto IAP have not been described in unfertilized ova and zygotes.

This paper gives details of the discovery of IAP in early zygotes of C57BL/6 mice, a strain with low predisposition to cancer, at the age of 5-7 h after mating.

EXPERIMENTAL METHOD

An electron-microscopic study was made of zygotes of C57BL/6 mice. Animals with a dated time of pregnancy were used. Embryos at the zygote stage, 5-7 h after mating, were removed from the female reproductive tract. Isolated oviducts were dissected on a watch glass in warm Whitten's medium, in which bicarbonate was replaced by Hepes. The zygotes were removed by flushing out the oviducts with medium by means of a medical syringe or by rupturing the oviduct with a fine needle at the site of the embryos. More than 70 zygotes were investigated.

Fixation, staining, and embedding of the zygotes in epoxide resins for electron microscopy were carried out by the usual methods. Serial ultrathin sections, after staining with a saturated solution of uranyl acetate (UA) in methanol or with a 1% solution of UA and lead citrate, were studied in the JEM-100B electron microscope with an accelerating voltage of 80 kV.

EXPERIMENTAL RESULTS

IAP were found in large numbers in serial ultrathin sections in all zygotes aged 5-7 h after fertilization which were studied (Fig. 1a). At this stage of development the zygotes have male and female pronuclei. IAP were recorded in different parts of the cytoplasm of the embryos. They are formed near the membrane of the smooth endoplasmic reticulum (ER) (Fig. 1b) and mature by budding into the lumen of the ER cisterns.

Some endogenous IAP are regularly spherical in shape with an external diameter of 70-75 nm. Their nucleoid consists of two concentric spheres with an external diameter of 50-60 nm and electron-optically translucent center 20-26 nm in diameter. The nucleoid is closely applied to the outer membrane of the virion. However, most IAP forming or already located inside the ER cisterns are not regularly spherical in shape but con-

Laboratory of Associated Infections, N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. Department of Embryology, M. V. Lomonosov Moscow University. (Presented by Academician of the Academy of Medical Sciences of the USSR V. M. Zhdanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 7, pp. 94-96, July, 1983. Original article submitted October 15, 1982.

 0007-4888/83/9607-0992 $07.50

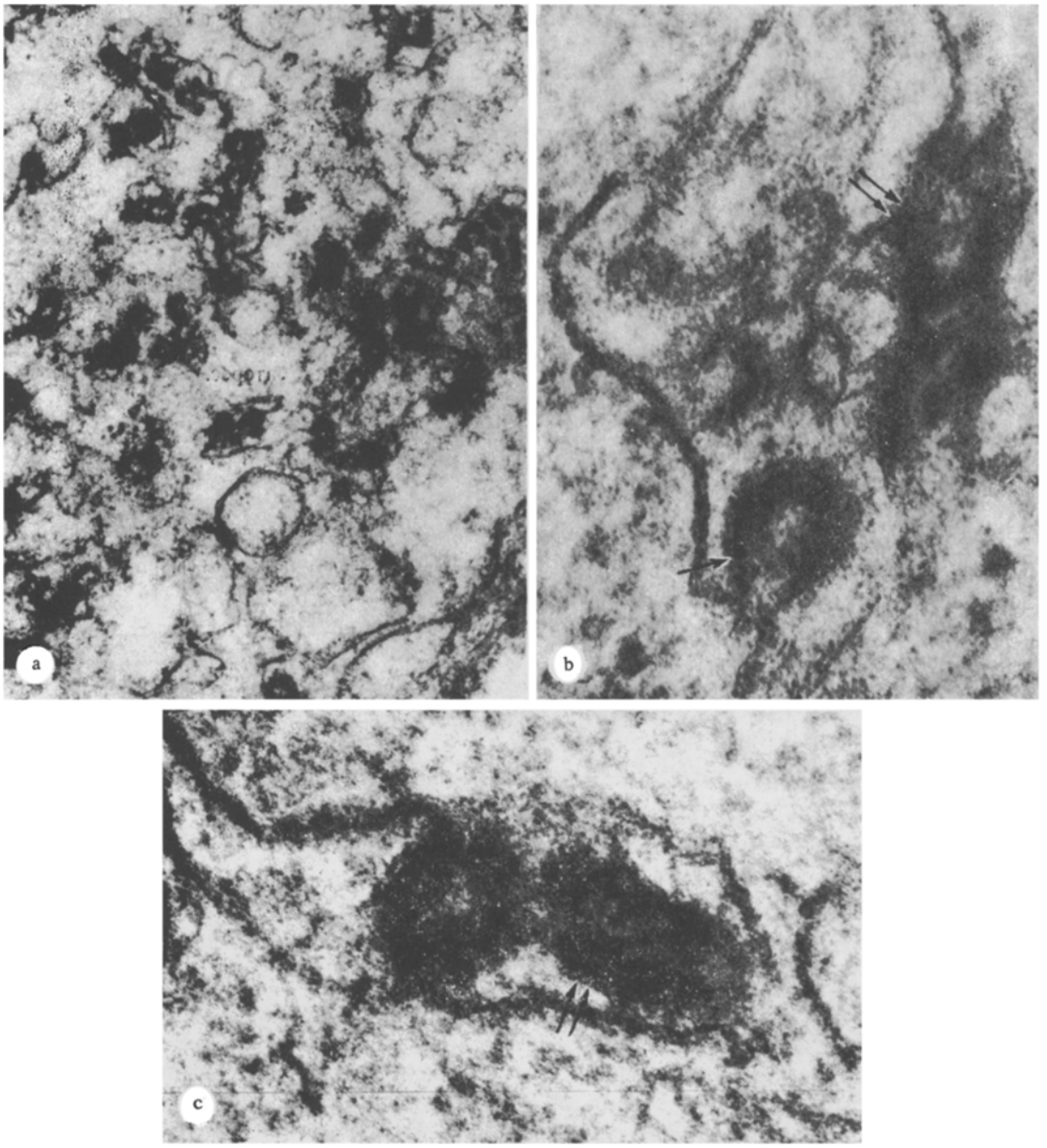

Fig. 1. Ultrathin sections through mouse zygote. a) Concentration of type A endogenous intracisternal particles. 80,000 ×; b, c) ordinary IAP formed near ER cistern (arrow), and abnormal IAP being formed or situated in lumen of ER (double arrows). 320,000 ×.

sist of separate fragments of nucleoids, connected by zones of lower density, forming hemispheres, bead-like shape, cylindrical formations, and other morphological anomalies (Fig. 1b, arrows).

IAP were often located in large vesicles of the Golgi complex, located both in the inner zones of the cell and close to the cell surface (Fig. 2). In the latter case IAP may also be seen in the immediate vicinity of the vitelline membrane of the zygote. In no case were virus particles found in the pronuclei or in the perinuclear space. No other types of endogenous murine viruses likewise were found in the present investigation.

Various workers have described four morphological variants of particles in oocytes and preimplantation embryos of different lines of mice, starting with the 2-4-cell embryo stage [7]. Three of them are similar in their submicroscopic organization to IAP, they are located in cisterns of the rough ER and differ only in size. The fourth type appears only at the blastocyst stage and, morphologically, belongs to the C type of murine retroviruses. Recently particles of yet another type have been described [11]; they have been called "early murine embryonic IAP" and designated by the letter ε (epsilon). They are found after the two-cell stage of development

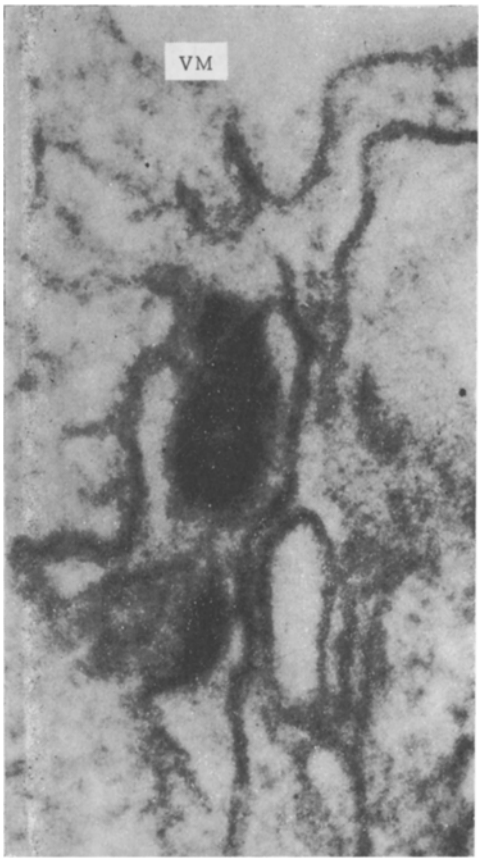

Fig. 2. IAP in vesicles of Golgi complex located in immediate vicinity of vitelline membrane (VM) of zygote. 250,000 ×.

and morphologically they are similar to the hamster endogenous R type of virus, but differ from it in the internal structure of the nucleoid and their smaller size.

The absence of data indicating discovery of endogenous viruses in zygotes was evidently not unexpected, for the zygote is a relatively resting stage where active primary biosynthesis is largely inhibited [1, 5]. This information, obtained previously by ultrastructural and biochemical methods, demonstrated the virtually complete absence of free ribosomes and rough ER in the zygote. Active nucleic acid and protein synthesis does not begin until the 4-cell embryo stage. Subsequent investigations showed that the early zygote contains a few free ribosomes, and we ourselves found for the first time initial stages of formation of a rough ER. Nevertheless, expression of virus-like particles, as we showed, took place in the smooth ER. The particles described above do not differ morphologically from classical IAP of mice, but the presence of a high percentage of anomalous formations may be linked with structural and biochemical features of the particular compartment of the zygote, which is a system of membranous formations – sacs, vesicles, and canals, united in the concept of "smooth ER." In the late stages of preimplantation development (from the 4-cell to the blastocyst stage), however, and also in normal and cancer cells of adult mice, IAP are formed mainly in the rough ER. Very recently IAP have been described in cisterns of ER in zygotes of four of six lines of mice studied [11], but not in line C57BL/6. Unfortunately, the absence of any mention of the age of the zygotes in the paper cited above prevents the drawing of any parallels with our own observations.

The role and functions of IAP are not yet understood. In the course of embryonic development the number of IAP falls gradually, and by the blastocyst and ovular cylinder stage their expression is practically reduced to zero. This inversely proportional dependence of IAP on the degree of differentiation of the embryo is of great interest for it reflects a rather special biological situation linked either with the undifferentiated state or, conversely, with the beginning of early differentiation.

Further investigations of early embryos of different mammals in the search for endogenous viruses may be of great interest.

LITERATURE CITED

1. V. I. Bogomolova, A. I. Golubitsa, A. I. Zhelezova, et al., Ontogenez, No. 1, 610 (1981).
2. W. Biczysko, M. Pienkowski, D. Solter, et al., J. Natl. Cancer Inst., 51, 1041 (1973).
3. W. Biczysko, D. Solter, C. Graham, et al., J. Natl. Cancer Inst., 52, 483 (1974).
4. R. A. Bowen, J. Natl. Cancer Inst., 65, 1317 (1980).
5. P. G. Calarco and E. U. Brown, J. Exp. Zool., 171, 253 (1969).
6. P. G. Calarco, Biol. Reprod., 12, 448 (1975).
7. D. G. Chase and L. Pikó, J. Natl. Cancer Inst., 51, 1971 (1973).
8. H. Sobis and M. Wandeputte, J. Natl. Cancer Inst., 61, 891 (1978).
9. S. S. Yang, P. G. Calarco, and N. A. Wivel, Eur. J. Cancer, 11, 131 (1975).
10. Y. Yotsuyanagi and D. Szöllösi, Biol. Cell., 39, 201 (1980).
11. Y. Yotsuyanagi and D. Szöllösi, J. Natl. Cancer Inst., 67, 677 (1981).

DIFFERENTIATION OF NEUROBLASTOMA CELLS UNDER THE INFLUENCE OF CYTOCHALASIN B

V. B. Kishkina, L. V. Domnina, Yu. M. Vasil'ev, and I. M. Gel'fand

UDC 616-006.487-018.15-02:615.277.3

KEY WORDS: neuroblastoma; cytochalasin B; cell differentiation.

Several lines of neuroblastoma cells capable of growth in culture have been described [2, 5, 6]. Undifferentiated and actively proliferating cells of these lines can be converted, under the influence of various factors (serum-free medium, dimethyl sulfoxide, analogs of cyclic nucleotides [6, 7, 8]) into differentiated cells, morphologically similar to neurons. Neuroblastoma cultures are thus a convenient object with which to study factors controlling differentiation of tumor cells.

The object of this investigation was to study the possibility of inducing differentiation of neuroblastoma cells by treatment with small doses of cytochalasin B (CB), an agent with selective action on the actin microfilament system. One of the results of destruction of microfilaments by CB is blocking of the last phase of mitosis, leading to the appearance of multinuclear cells. It was found previously that the multinuclear state leads to partial normalization of the morphology of transformed cells of another type, namely fibroblasts of the L line [1].

EXPERIMENTAL METHOD

A culture of mouse neuroblastoma cells of clone N-18-A, line C-1300 [2, 5] was grown at 37°C on Eagle's medium with 10% neonatal calf serum and gentamycin (80 units/ml). For the experiments the cells were planted on coverslips in the proportion of 10,000 cells to 1 ml medium.

CB in a dose of 1.8 μg/ml (from Serva, USA) was added to the culture medium 24 h after seeding. The cells were incubated with CB for three days, then transferred for 1-4 days to medium without CB. Control cultures were incubated throughout the experiment (7-8 days) without CB in normal medium with serum. Cultures grown for 7-8 days in serum-free medium served as the second control. The morphology of the living cells and their contacts with the substrate were investigated by phase-contrast and interference-reflection microscopy [4].

A. N. Belozerskii Interfaculty Research Laboratory for Problems in Molecular Biology and Bioorganic Chemistry, M. V. Lomonosov Moscow University. Laboratory of Mechanisms of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 7, pp. 97-99, July, 1983. Original article submitted December 17, 1982.