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Distribution of Intracisternal A Particles in Mouse Teratocarcinoma-derived Cell Lines*

MARTINE CANIVET,† JACQUELINE LASNERET, LAURENT DIANOUX, ALBERTO ROSETO and JORGE PERIES

Unité 107 *INSERM, Virologie des Leucémies, Hôpital Saint-Louis,* 2 *place du Docteur A. Fournier,* 75 475 *Paris Cedex* 10, *France*

Abstract—*The distribution of intracisternal A particles in several mouse teratocarcinoma derived cell lines, was studied by electron microscopy. Three of these lines consist of embryonal carcinoma stem cells. The presence of intracisternal A particles was quantified by two different criteria:* (1) *by counting the cells containing at least one particle;* (2) *by counting the total number of particles in* 100 *cellular sections. These studies indicate that neither C nor B particles were present in any of the lines, while intracisternal A particles appeared in all of them. No clear relationship between the presence or the number of this type of particles with either the state of differentiation or the potentiality of cells, could be demonstrated. However, the observation that the number of particles was strongly diminished in a differentiated cell line derived* in vitro *from an EC cell line suggests a role of intracisternal A particles in the regulation of the undifferentiated cellular state.*

INTRODUCTION

Mouse intracisternal A particles are virus-like structures which are found either within the cisternae of the endoplasmic reticulum or budding at the reticulum membrane [1]. They have a spherical form and consist of two concentric shells surrounding a relatively electron-lucent core. They measure between 70 and 100 nm in diameter and possess a high molecular weight RNA (70S) [2], a major structural protein (73,000 Daltons) [3] and an endogenous RNA-dependent DNA-polymerase activity [4, 5]. Specific intracisternal A particles genetic information is integrated as DNA in mouse cellular genome in the form of repetitive sequences [6, 7]. Intracisternal A particles have been described in a large number of mouse tumour cells and in several types of normal tissue [8]. Their biological significance is unknown, but their programmed appearance in the early stages of mouse embryonic development [9], and their disappearance as differentiation is achieved, plead in favour of an eventual role in embryogenesis and cellular differentiation [10–12].

Mouse teratocarcinoma has been proposed as a valuable model for the study of mouse development and differentiation [13] because they contain embryonic like-cells, called Embryonal carcinoma cells (EC cells) which are comparable to the primitive stem cells of early embryos. EC cells and differentiated cell lines have been derived *in vitro* [13–15] from this type of tumor and they are presently used as experimental material in studies on various aspects of cellular differentiation. This communication describes the results of experiments concerning the distribution of intracisternal A particles in some of these cell lines and the eventual correlation between them and the state of differentiation or the potentiality of cells.

MATERIALS AND METHODS

Cells

Table 1 summarises the main characteristics of the cell lines used in this study. They have been kindly provided by F. Jacob (Institut Pasteur, Paris) and maintained according to the methods described by his group [14, 15].

The PCC4 line contains multipotent EC cells which differentiate exclusively *in vivo* [15]. The PCC3 line is also mostly composed of multipotent EC cells [14, 15]. However,

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†To whom requests for reprints should be addressed.

Table 1. *Cell lines*

Cell line	Type of cells	Potentiality	Alkaline phosphatase*†	Acid phosphatase*†	F9 antigen*‡	PCC4 antigen*§	Viral susceptibility*¶
PCC4	EC	Multipotential *in vivo*	80	0	50	90	0
PCC3	EC	Multipotential *in vivo* and *in vitro*	95	2	50	90	3
PCC6	EC	Nullipotential	79	2	Nd‖	Nd‖	0
PCC3d$^+$	Pluritissular	—	0	85	0	0	80
Pys II	Endodermal	—	Nd‖	Nd‖	Nd‖	0	50
PCD1	Myocardial	—	0	100	0	0	85
PCD3	Fibroblastic	—	0	100	0	0	80

*%positive cells.
†Histochemical test.
‡Cytotoxicity.
§Immunofluorescence.
¶P30 Immunofluorescence.
‖Nd = Not done.

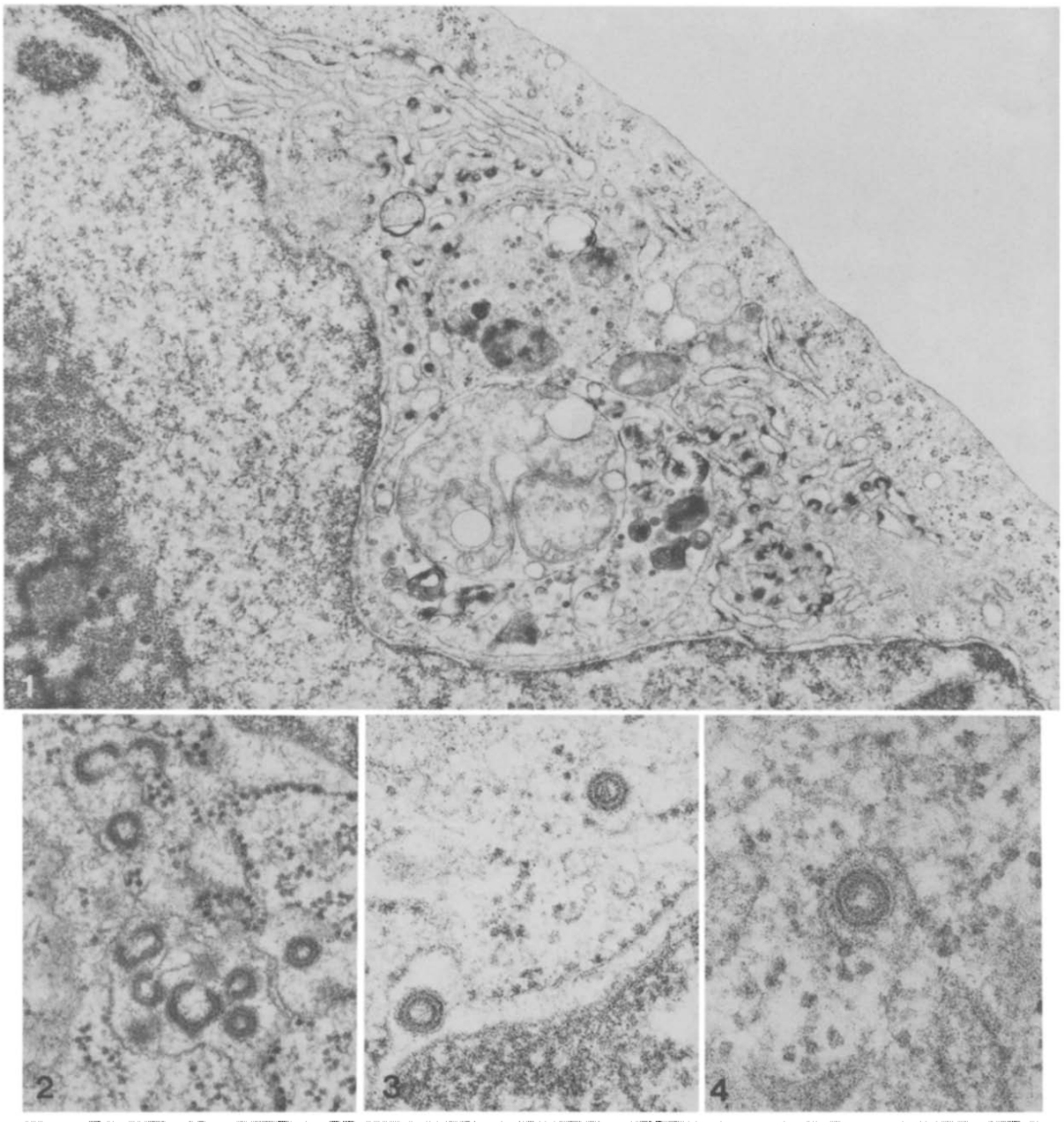

Fig. 1. *Magnification* 24,500. *PCC6 cells. Very numerous intracisternal A particles, mostly budding in the light of endoplasmic reticulum.*

Fig. 2. *Magnification* 66,500. *PCC3 cells. Intracisternal A particles are grouped in circle lines and budding in the light of cisternae.*

Fig. 3. *Magnification* 87,500. *PCD3 cells. Isolated intraciternal A particle is present in the perinuclear space.*

Fig. 4. *Magnification* 120,000. *Structural detail of a intracisternal A-type particle.*

unlike PCC4 cells, they differentiate *in vitro* and some heterogeneity may be observed through replating. In our experiments we used only freshly cultured cells and less than 5% of the PCC3 cellular population present morphological (optical and electron microscopy) [13], histochemical (phosphatases) [14], immunological (F9 and PCC4 antigens negativity) [16] or biological (susceptibility to murine type C viruses) [17, 18] markers of differentiation. PCC6 is also an EC cell line but its cells are nullipotent and give rise, after infection, in syngenic mice to tumors containing almost exclusively EC cells. The PCC3d$^+$ line has been derived in our laboratory from PCC3 after differentiation *in vitro* by culture without replating [14]. The majority of these cells are fibroblastic. Other cell types: lipid, skeletal muscle and epithelial, are also present in PCC3d$^+$ but in a much lower number; neither EC cells nor endodermal cells are detectable in this line. PCC3d$^+$ is non tumorigenic when injected to mice. Pys II is a differentiated endodermal cell line [19]. PCD1 and PCD3 are differentiated lines. The former is composed of myocardial myoblastic cells, the latter of fibroblastic like-cells [20]. Primary cultures of 129 and A/He mice fibroblasts, prepared by standard methods, were used as controls.

Electron microscopy

Cells were examined in a Philips 301 electron microscope. They were fixed in glutaraldehyde, post-fixed in osmium and embedded in Epon before being double-stained with uranyl acetate and lead citrate. At least 100 sections were systematically scanned for each cellular sample. The ultrastructural characteristics were adopted as criteria to evaluate the differentiation state [13] and to characterize the cytological type. Care was taken to avoid counting the same or too proximal fields. Particles were recognised by their morphology, size and intracisternal localisation. They were quantified following two different criteria: (i) the percentage of cells showing at least one particle, (ii) the total number of particles existing in 100 cellular sections.

RESULTS

When the seven differentiated lines and the primary explanted embryo fibroblasts were examined by electron microscopy, neither type C nor B particles were detected. The primary embryo fibroblasts cultures were also negative for intracisternal A particles. However, typical intracisternal A particles were observed in all the teratocarcinoma lines. Most of them were found budding in the membranes of the endoplasmic reticulum (Figs. 1 and 2), but in many cases free particles were also detected in cisternae (Figs. 3 and 4).

The number of positive individual cells containing at least one intracisternal A particle varied with the different lines. Table 2 summarizes this variability. The highest percentages of positive cells (more than 50% of examined cells) were found in PCC6 and PCD3 lines. PCC3, PCC3d$^+$, PCD1 and Pys II showed less than 50% of positive cells. The PCC4 cell line contained only few cells possessing particles; scarcely 1% of cells were positive.

Table 2 also shows the results obtained when the evaluation of the amount of particles was based on the counting of the total number of particles per 100 cellular sections. One observes that PCC6 and PCD3 cell lines contain the highest number of particles. PCC3, PCC3d$^+$, PCD1 and Pys II showed less virus-like particles and PCC4 practically none. Observing the Table, it is interesting to

Table 2. Quantification of intracisternal A particles (IAP)

Cell line	% of IAP positive cells	Total number of particles per 100 cellular sections
PCC6	80	TNTC*
PCC3	35	107
PCC3d$^+$	27	34
PCD1	42	90
PysII	46	94
PCC4	0.25	<1
Mouse embryo fibroblasts (MEF)		
129 MEF	0	0
A/He MEF	0	0

*Too numerous to be counted.

point out that even if the number of cells containing at least one particle is practically the same in both PCC3 and its differentiated derivative PCC3d$^+$, the total amount of particles per 100 cellular sections is significantly lower in the latter.

DISCUSSION

Intracisternal A particles have never been observed in mouse unfertilized eggs and zygotes but they have been described in considerable number in late 2, 4 and 8 cell stage embryos from various mouse strains [21]. The amount of particles was practically the same in all these three situations. However, in blastocysts and egg cylinders the number of particles decreases considerably, as if they were modulated in parallel with the evolution of the embryogenesis. They have also been described by Teresky *et al.* [22] in embryoid bodies derived from 129 teratocarcinomas taken directly from the animal or cultured *in vitro* for several months. In this last system, the particles were found predominantly in the outer endodermal cells of the embryoid bodies.

Our report provides the first systematic ultrastructural study of the distribution of intracisternal A particles in teratocarcinoma derived cell line cultures *in vitro*, and, in addition, it supplies information about the absence of C and B type particles in this kind of material. Even if the number of cell lines examined is far from being enough to draw definitive conclusions, our results suggest that the distribution of intracisternal A particles, as evaluated by transmission electron microscopy, fails to be clearly correlated either to the state of differentiation or to the potentiality of the cells. However, our observation of the lower quantity of intracisternal A particles present in the differentiated PCC3d$^+$ cells compared to undifferentiated PCC3 cells, suggests, as has otherwise been proposed [9–11], that the expression of these particles may be related to some particular biological situation in rapport with the maintenance of the state of undifferentiation and/or with early stages of differentiation. The high expression of intracisternal A particles in nullipotent PCC6 cells, where they are too numerous to be counted, may be in favor of the former hypothesis. Additional experiments using more accurate biochemical and immunological techniques to evaluate the expression of intracisternal A particles are necessary to answer this question.

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