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The nature of the 'nucleolus precursor body' in early preimplantation embryos: a review of fine-structure cytochemical, immunocytochemical and autoradiographic data related to nucleolar function

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Summary

In mammals, the restoration of rRNA transcription after fertilisation is accompanied by a gradual differentiation of the nucleolar structure by a process called embryonic nucleogenesis. During cleavage, the nucleolar components appear sterically related to a class of nuclear bodies already detectable in pronuclei. These structures, due to their apparent function as centres of nucleolus formation, have been designated nucleolus precursor bodies (NPBs). It was found recently not only that the size and morphology of the NPBs differ among mammalian species, but that the pattern of embryonic nucleologenesis and even the molecular composition of different NPB compartments vary from one species to another. Accordingly we assumed that at least two definitely different types of NPBs exist, namely the mouse-type NPB and cow-type NPB. In the mouse-type NPB, the original compact material of the NPB remains detectable in the early functional nucleolus. This NPB core does not contain DNA or typical Ag-NOR nucleolar proteins. At the onset of rRNA transcription, the nucleolonema is formed at the periphery of the NPB. The cow-type NPB shows a homogeneous distribution of typical nucleolar proteins throughout its body from the pronucleolar to the early 8-cell stage. At the beginning of rRNA transcription, the cow-type NPB is penetrated by perinucleolar DNA and rRNA synthesis is detectable deep inside the nucleolus. In this case, the entire NPB is readily transformed into a typical nucleolus. These processes are recognisable using fine-structure analysis of preimplantation mammalian embryos. For this reason this approach is often used as a method of evaluating the state of experimental embryos; in such studies, the species differences must be taken into account.

Keywords: Cleaving embryo, Electron microscopy, Mammals, Nucleologenesis, rDNA

Introduction

The restoration of transcription in early preimplantation mammalian embryos occurring during the initial cleavage stages following fertilisation (for review see Telford *et al.*, 1990) is linked with changes in chromatin structure (Thompson, 1996; Wolfe, 1996). Correlatively a restructuring of the nuclear morphology occurs, which has been studied mostly by electron microscopy (see reviews on the mouse by Geuskens & Alexandre, 1984; the cow by Betteridge & Fléchon, 1988 and Kopečný & Niemann, 1993; the pig by Tománek *et al.*, 1989; and man by Tesařík, 1988). In all these mammalian species, the re-establishment of the cell nucleolus (the nuclear domain specialised in the transcription of ribosomal DNA) is correlated with the onset of rRNA transcription. Although often studied, this process, called embryonic nucleologenesis (in contrast to the somatic cell nucleogenesis that occurs at each cell cycle), remains a subject of debate. It has been observed that mammalian zygotes already possess several intranuclear bodies, which represent a kind of nucleolar 'anlage' since they are the structural entities that are directly involved (albeit by amazingly different species-specific ways) in the differentiation process of the nascent nucleolus. The differences in shape and size of these bodies and

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the particular pattern of nucleologenesis in different species have been compared by Kopečný (1989), on the basis of fine-structure autoradiographic observations.

The precursor bodies of functional nucleoli were first supposed to represent already formed nucleoli, albeit of a different texture. They were then called 'compact nucleolus' (Faken & Odartchenko, 1980) or 'primary nucleolus' (Geuskens & Alexandre, 1984). To underline the difference with a functional nucleolus, they were named 'nucleolus-like body' (Takeuchi & Takeuchi, 1982; Tesařík *et al.*, 1986) or 'prenucleolar body' (Biggiogera *et al.*, 1990). Based on broad evidence discussed in the present review, we suggest that, from the functional point of view, these organelles should provisionally be designated 'nucleolus precursor body' (NPB) due to their established generic relation with the nucleolus. This term was proposed earlier (Kopečný *et al.*, 1989*a*, 1991) and has already been used in recent studies on the nuclei of early mammalian embryos (Biggiogera *et al.*, 1994; Cuadros-Fernandez & Esponda, 1996; Baran *et al.*, 1996; Kaňka *et al.*, 1996).

NPBs represent an obvious feature of the nuclear morphology of mammalian embryos. Therefore, NPBs have been used, among other criteria, as an objective guideline to indicate the effect of mammalian embryo micromanipulation (for reviews see Kaňka *et al.*, 1991; Kopečný & Niemann, 1993; Pavlok *et al.*, 1993; Fulka *et al.*, 1996). However, the exact interpretation of these observations from a morpho-functional point of view requires a more complete understanding of the composition of the NPBs and of their interaction with the parts of the genome involved in the transcription of rRNA – the nucleolus organizing regions (NORs). This field of research is worth developing, as the mammalian embryo itself is an alternative model to somatic cells in basic research on nuclear and nucleolar biology.

As part of the interest of embryologists in the onset of zygotic gene expression, much work has been undertaken by cytochemical and immunocytochemical techniques at the light and electron microscope level on nucleolar structures of cleaving mammalian embryos (mouse: Fakan & Odartchenko, 1980; Takeuchi & Takeuchi, 1986; Biggiogera *et al.*, 1990, 1994; Baran *et al.*, 1993, 1995; rabbit: Baran *et al.*, 1997; pig: Kopečný *et al.*, 1996*a*; cow: King *et al.*, 1988; Kopečný *et al.*, 1991, 1996*b*; Baran *et al.*, 1996; goat: Chartrain *et al.*, 1987; Kopečný *et al.*, 1996*b*). While these studies give some insight into the localisation of different nuclear and nucleolar proteins in early preimplantation mammalian embryos, a new enigma has emerged about the concept of the NPB. There is a striking species-specific difference in the molecular composition, that is in the distribution of the components of the transcription machinery, detected in NPBs. On one hand, a group of mammals (rodents and pigs) lack Ag-NOR proteins (proteins typical of nucleoli and revealed by a specific silver staining: see recent data in Roussel *et al.*, 1996) and also DNA in the core of the NPB. On the other hand, in bovine and caprine embryos, the NPB is already stained in pronuclei by this silver cytochemical method, indicating the presence of some typical nucleolar proteins in the earliest stage of the NPB. Moreover, contrary to rodents, the bovine and human NPB is penetrated by DNA from the beginning of nucleologenesis. In the present review we discuss these points and attempt to give a synthetic view of these phenomena.

Mouse-type NPB

In the mouse (Calarco & Brown, 1969; Fakan & Odarchenko, 1980), as well as in several other rodents (rat: Takeuchi & Takeuchi, 1982; review Antoine, 1989; hamster: Uehara & Yanagimachi, e.g. 1977; Naish *et al.*, 1987), in the rabbit (references in Kaňka *et al.*, 1996), but also in the pig (Szöllösi & Hunter, 1973; Tománek *et al.*, 1989; Laurinčík *et al.*, 1995) soon after pronucleus formation, a very prominent nuclear body appears as a sphere sharply delineated from the surrounding nucleoplasm (cf. Figs. 1 and 3). As seen by electron microscopy, this sphere is composed of a very dense meshwork of thin fibrils. The differentiation of functional nucleolar structures is strictly limited to its periphery, so that a dense core of the NPB is still present in the activated nucleolus. By a process that remains poorly understood (associated with surface 'decondensation': Antoine, 1989), Ag-NOR stained material first appears at the periphery of this type of NPB, being localised in so-called lenticles (Takeuchi & Takeuchi, 1986, see Fig. 8; Biggiogera *et al.*, 1990). Later, fibrillar centres and then a typical nucleolonema appear, both restricted to the periphery of NPBs (Geuskens & Alexandre, 1984; Tománek *et al.*, 1989, see Fig. 2). The core of the original NPBs remains compact and is still detectable in the following cleavage stages up to the morula in mouse (Geuskens & Alexandre, 1984) and in pig (Tománek *et al.*, 1989, see Fig. 2), and according to a general consensus

Figures 1 and 2 Absence of participation in RNA synthesis of the compact material of mouse-type nucleolus precursor body (NPB) as shown by fine-structure autoradiography. **Figure 1** Newly synthesised nucleic acids are not localised within the dense mass of a NPB of a hamster pronuclear embryo cultured for 10 h in the presence of [8-^{3}H]adenosine. ne, nuclear envelope. From Kopečný *et al.* (1995). Scale bar represents 0.5 μm. **Figure 2** The central compact mass of the mouse-type NPB remains definitively unlabelled as seen in a fine-structure autoradiogram of a morula-stage pig embryo cultured for 20 min in the presence of [5-^{3}H]uridine. In contrast, the dense fibrillar component of the already functional nucleolus shows high uridine incorporation. ne, nuclear envelope. Modified from Tománek *et al.* (1989). Scale bar represents 0.5 μm.

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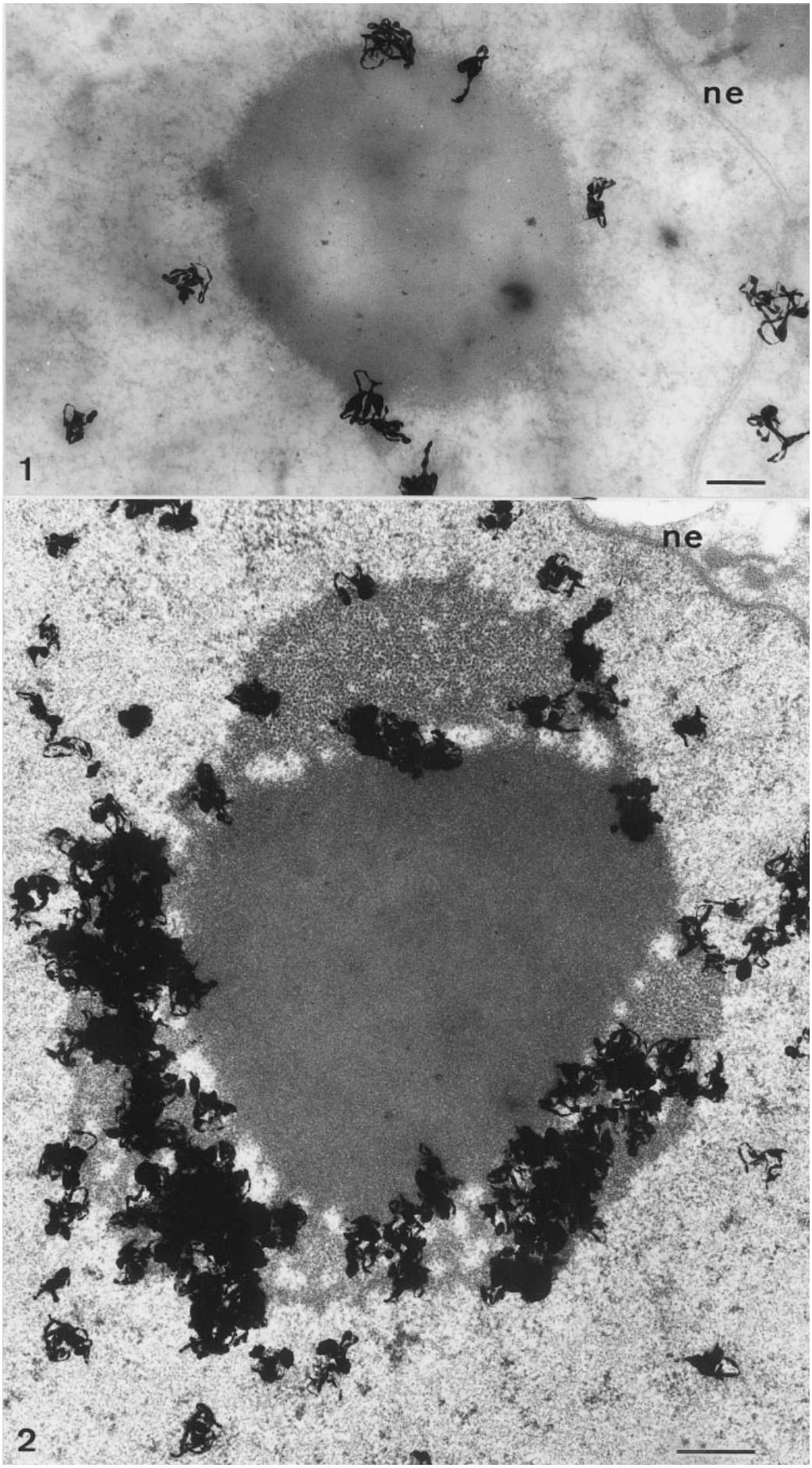

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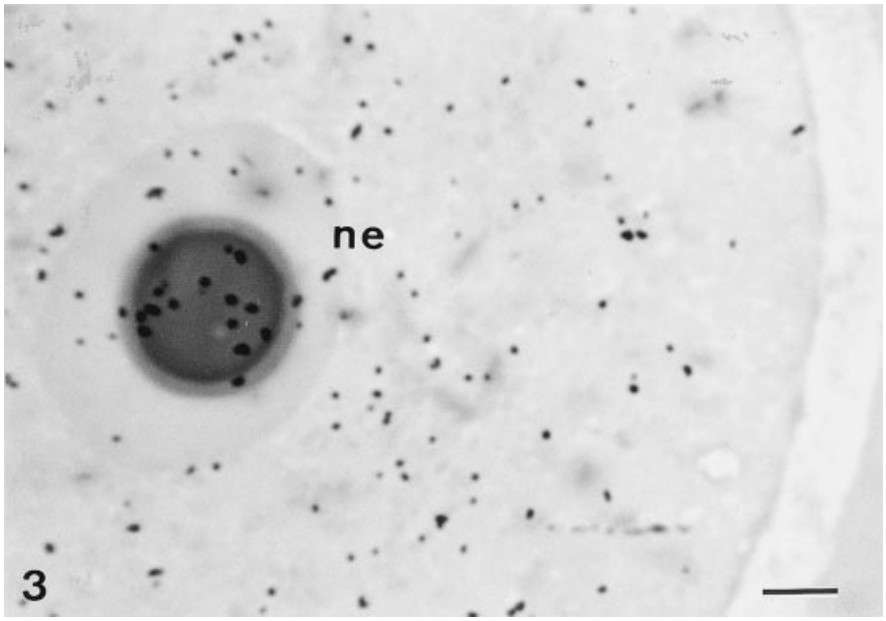

Figure 3 In contrast to a total absence of newly synthesised RNA in the mouse-type NPB shown in Figs. 1 and 2, radioactivity was detected by light microscope ARG in pronuclear NPBs of mouse embryos developed *in vitro* from oocytes previously cultured in the presence of [5-^{3}H]uridine. This labelling probably corresponded to long-lived, 'maternal' RNAs. ne, nuclear envelope. From Kopečný *et al.* (1995). Scale bar represents 2 μm.

it is never silver-stained (mouse: Takeuchi & Takeuchi, 1986; Biggiogera *et al.*, 1990; rat: Takeuchi & Takeuchi, 1982). There is also general agreement that the core of the mouse-type NPB does not contain DNA either before or during the onset of rRNA transcription and nucleolonema differentiation (Faken & Odartchenko, 1980; Baran *et al.*, 1993; Biggiogera *et al.*, 1994; Kopečný *et al.*, 1995). The absence of DNA was also confirmed recently in the rabbit NPB by the anti-DNA labelling and terminal deoxyinucleotidyl transferase techniques (Baran *et al.*, 1997). The appearance of the nucleolonema and hence of rRNA transcription only at the surface of the NPB was also supported by immunoelectron microscope detection of typical nucleolar proteins exclusively at the NPB periphery (Baran *et al.*, 1995; Cuadros-Fernandez & Esponda, 1996).

There are, however, reports of some nucleolar proteins localised in the core of the mouse NPB (Biggiogera *et al.*, 1990, 1994). Other immunoelectron microscopic observations suggest the presence of a class of non-nucleolar nucleoplasmic proteins involved in pre-mRNA splicing in the mass of the NPB in the mouse (Biggiogera *et al.*, 1994) or in the pig (Kopečný *et al.*, 1996*a*). In these studies, the main spliceosomal components detected were small nuclear ribonucleoproteins (snRNPs) and also snRNA. It was suggested that all these molecules may be of maternal origin, since there is no accumulation either of newly synthesised RNA (Geuskens & Alexandre, 1984) or of nucleic acids in general (Kopečný *et al.*, 1995, see Fig. 1) in the NPB of the early mouse embryo or early pig embryo (Tománek *et al.*, 1989). In fact, [5-^{3}H]uridine-labelled macromolecules were detected by autoradiography only in NPBs of (parthenogenetic) embryos originating from oocytes pre-cultured in medium enriched with this precursor (Kopečný *et al.*, 1995, see Fig. 3). A possible kinship of the mouse-type NPB to the so-called sphere organelle (Gall *et al.*, 1995; Roth, 1995) was discussed recently (Kopečný *et al.*, 1996*c*). Sphere organelles are prominent bodies of the amphibian oocyte nucleus that occur free or attached to specific chromosomal loci and containing snRNPs and hence belong to a broad class of intranuclear bodies called snurposomes. Both organelles contain similar proteins (especially spliceosomal components), and probably display a comparable pattern of RNA enrichment in oocytes of amphibians and mammals (Gall & Callan, 1989; Motlík *et al.*, 1984). Thus the mouse-type NPB may also possibly play a role in snRNP storage.

Cow-type NPB

In the cow, as well as in the goat, the NPBs in early pronuclei are quite inconspicuous (Crozet, 1984; Chartrain *et al.*, 1987; Hytell *et al.*, 1988; review in Laurinčík *et al.*, 1996; Kopečný *et al.*, 1989*a*, 1991, 1996*b*). Their differentiation into functional nucleoli concerns the whole organelle. In other words the bovine NPB is completely transformed into a typical nucleolus con-

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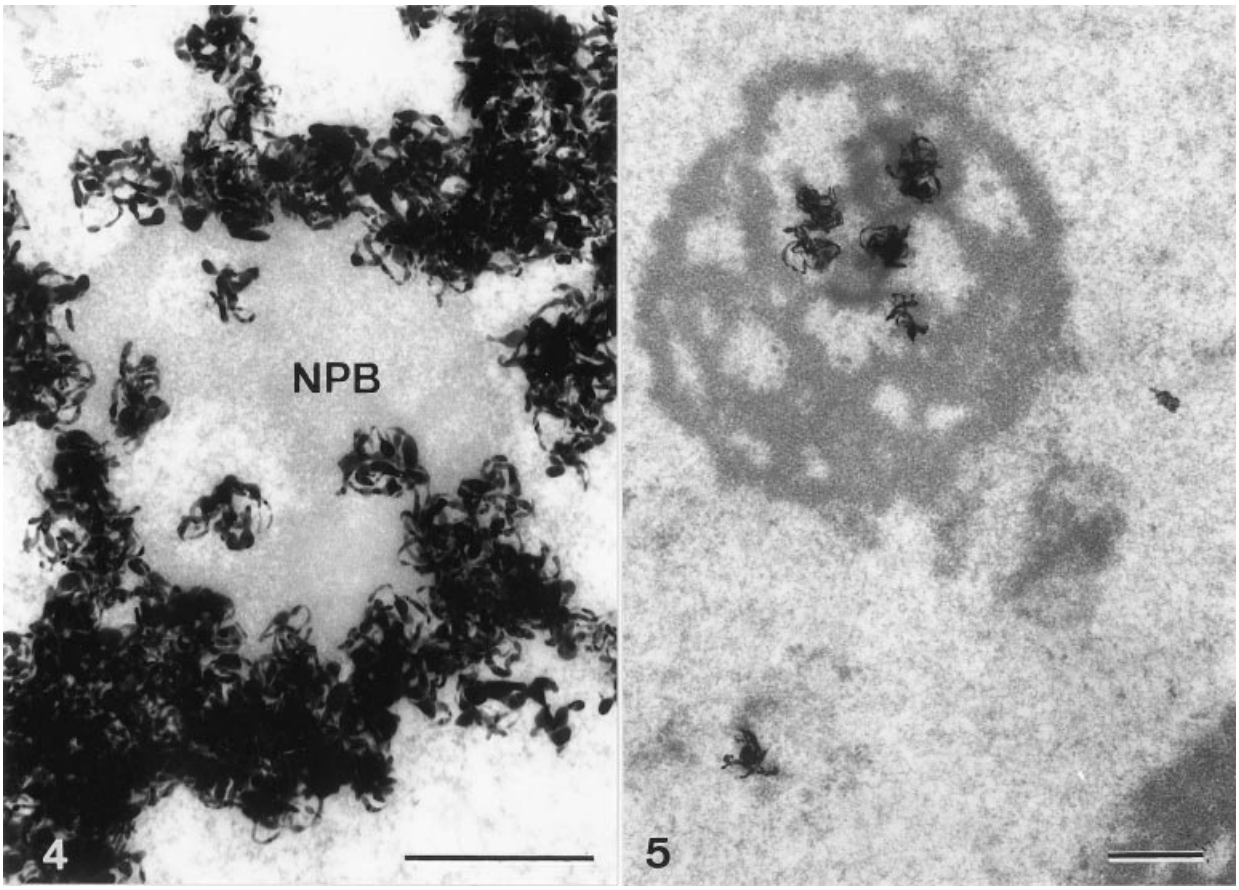

Figures 4 and 5 Autoradiographic demonstration of the localisation of newly synthesised nucleic acids in the central area of the cow-type NPB. **Figure 4** At the onset of major RNA transcription in the late 8-cell bovine embryo, the mass of its NPB is penetrated by newly synthesised replicated DNA, detectable by a very intense labelling by [methyl-^{3}H]thymidine of chromatin otherwise encapsulating the NPB. From Kopečný *et al.* (1989*b*). Scale bar represents 0.5 μm. **Figure 5** The entire cow-type NPB is rapidly transformed into a functional nucleolus at the end of the 8-cell stage. The typical nucleolar morphology emerged in the central area of the former NPB and [5-^{3}H]uridine fine-structure autoradiography showed intense labelling of the dense fibrillar component. From Kopečný *et al.* (1989*a*). Scale bar represents 0.5 μm.

taining no evident residual mass (Camous *et al.*, 1986; Kopečný *et al.*, 1989*a*, see Fig. 5; Kopečný *et al.*, 1991). The formation of a nucleolus from a NPB is concomitant with a deep DNA penetration into its core (Kopečný *et al.*, 1989*b*, see Fig. 4; Baran *et al.*, 1996). A similar situation has been described in the human embryo (Tesařík *et al.*, 1986, 1987).

The presence of argentaffin proteins, supposed to be typical of nucleoli, was detected in both cow and goat embryos very early in development (in pronuclei and 2-cell embryo nuclei), the whole mass of the NPB being stained uniformly (Kopečný *et al.*, 1996*b*, see Fig. 6). A similar homogeneous distribution of nucleolar protein C 23 was detected by Baran *et al.* (1996) in early 8-cell bovine embryo NPBs (Fig. 7). On the other hand, snRNPs were not detected in cow NPBs (Kopečný *et al.*, 1991), using exactly the same antibodies and techniques used to detect these spliceosomal nucleoproteins in the NPBs of mouse and pig, as discussed earlier. It is possible, however, that the cow-type embryo possesses a special class of nuclear bodies enriched in sRNPs (Kopečný *et al.*, 1996*b*), in addition to interchromatin granules (IGs), generally known to contain snRNPs. This type of body also occurs in mouse and pig (Tománek, 1989; Kopečný *et al.*, 1996*a*); however, they are very small and hence mostly escaped attention.

The cow-type NPB may probably be assigned to an inactive nucleolar structure, containing nucleolar proteins but lacking rDNA. Such structures were called 'dots' in cells lacking ribosomal genes and considered as nuclear bodies in which the nucleolar proteins are not assembled in the same way as in a functional nucleolus. Their redistribution inside these particular nucleolar compartments into normal nucleoli is probably induced by ribosomal genes and ongoing rRNA synthesis (Hernandez-Verdum *et al.*, 1991). In fact, the successive appearance of individual typical nucleolar proteins, once more within the whole body of the NPB,

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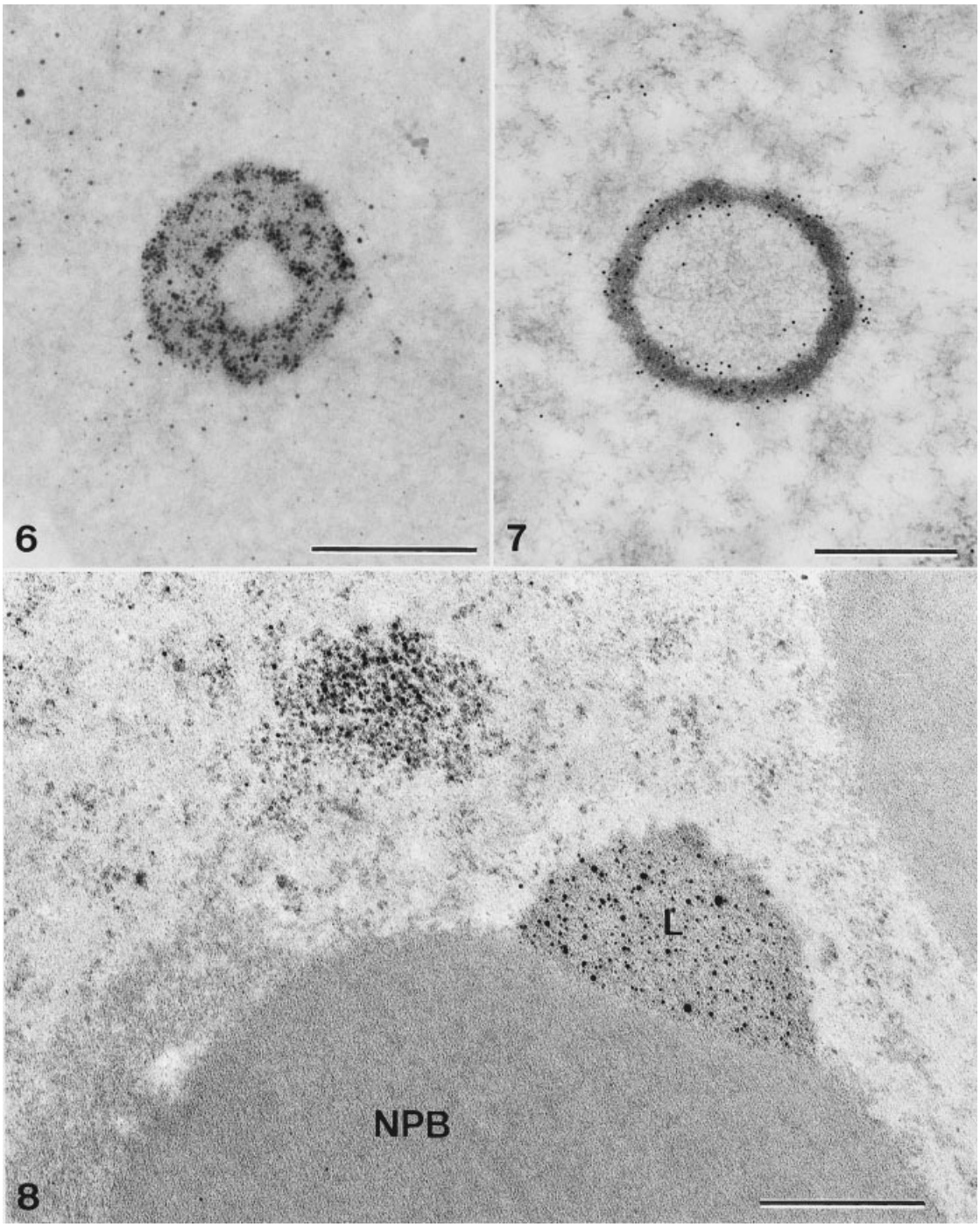

Figures 6–8 Difference in localisation of typical nucleolar proteins in cow-type versus mouse-type NPBs. **Figure 6** In the cow-type NPB the argentaffin nucleolar proteins (Ag-NOR proteins) are homogeneously distributed in the mass of the NPB as shown by specific silver staining of a NPB from a 2-cell bovine embryo. From Kopečný *et al.* (1996*b*). Scale bar represents 0.5 µm. **Figure 7** A similar homogeneity in the distribution of a typical necleolar protein C23 was detected by immunoelectron microscopy in a cow-type NPB from an early 8-cell bovine embryo. From Baran *et al.* (1996). Scale bar represents 0.5 µm. **Figure 8** Ag-NOR proteins are never detected in a mouse-type NPB, although they are revealed in a surface protrusion called a lenticle (L) and supposed to represent the material akin to the future fibrillar centre of the nucleolus. This situation is shown here in a 2-cell mouse embryo. From Takeuchi & Takeuchi (1986). Scale bar represents 0.5 µm.

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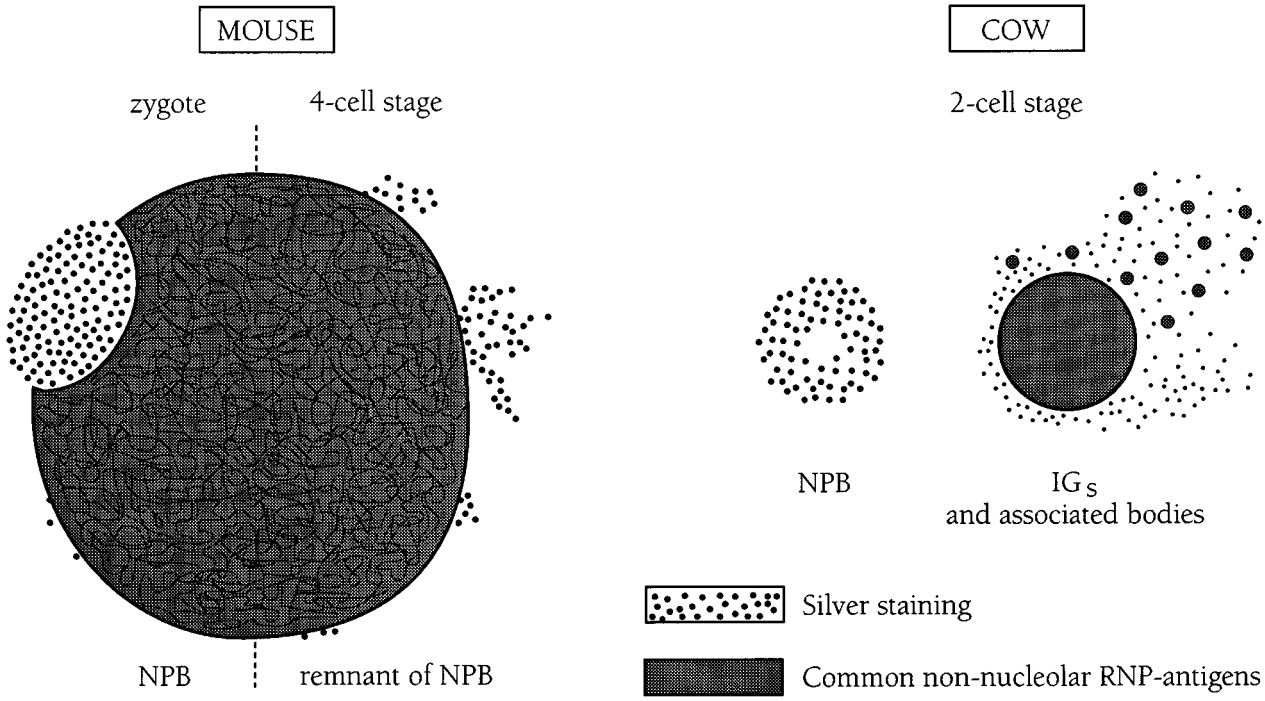

Figure 9 Schematic drawing recapitulating the basic differences in the distribution of nucleolus-specific proteins and some non-nucleolar antigens in NPBs occurring in early cleavage stages in the mouse or the cow (in relative size). In the mouse-type NPB, typical (argyrophilic) nucleolar proteins are detected only in the periphery of the NPB from the earliest stages of cleavage (cf. Fig. 8) up to an already functional nucleolus (cf. Fig. 2). In the cow-type NPB, similar nucleolar proteins are detected in the entire NPB from the earliest stages of cleavage (cf. Fig. 6) up to the beginning of the 8-cell stage (cf. Fig. 7). In parallel note the distribution of non-nucleolar antigens such as small nuclear ribonucleoproteins (snRNPs) in interchromatin granules (IGs) and associated nuclear bodies, as discussed in the text.

has been shown in the course of nucleolus differentiation in the bovine embryo (Baran *et al.*, 1996).

Conclusion

In conclusion, the term nucleolus precursor body is justified from an ontogenetic point of view considering that the first morphological features of nucleolar differentiation and function are sterically linked with this 'anlage'. On the other hand, the NPB as seen in the first phases of nucleolus formation in the mouse versus the cow, represents a quite different entity as far as its molecular composition is concerned (Fig. 9).

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