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Nuclear Bodies Are Usual Constituents in Tissues of Hibernating Dormice

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ABSTRACT

In previous studies we demonstrated in several tissues of the hazel dormouse *Muscardinus avellanarius* that during hibernation cell nuclei contain particular structural constituents absent in euthermia. In the present study we examine the same tissues in euthermic and hibernating individuals of the edible dormouse *Glis glis* in order to investigate possible modifications of nuclear structural constituents occurring during hibernation in this species.

Edible dormice were captured in the wild and maintained in an external animal house. Samples of liver, pancreas, brown adipose tissue and adrenal cortex were taken from three hibernating and three euthermic animals and processed for resin embedding. Ultrastructural and immunocytochemical studies were carried out on cell nuclei of these tissues.

The most evident feature of cell nuclei of hibernating dormice was the presence of several nuclear bodies, namely fibro-granular material, amorphous bodies, coiled bodies, perichromatin granule-like granules and nucleoplasmic fibrils, the distribution of which was peculiar to each tissue. No one of these constituents was detectable during euthermia. Immunocytochemical analyses revealed that they contain some splicing factors.

Apart from some differences, maybe due to the different characteristics of lethargy, the nuclear bodies found in edible dormice were morphologically and immunocytochemically similar to those previously described in the same tissues of hazel dormice. They therefore seem to be strictly correlated to the hibernating state. If they represent storage and/or assembly sites of splicing factors to be rapidly used upon arousal, they could represent a usual structural feature in cells of hibernating species. Anat Rec 254:389–395, 1999.

Key words: hibernation; cell nucleus; nuclear bodies; dormouse; electron microscopy; ultrastructure; immunocytochemistry

Hibernation represents for small mammals a peculiar type of adaptation to the environment. During the cold season, when the unfavourable habitat conditions make survival problematic, hibernators are able to drastically reduce their energy needs by entering long phases of lethargy characterised by low body temperature and mini-

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mum physiological and metabolic rate (for reviews see i.e. Hoffman, 1964; Lyman et al., 1982; Wang, 1987; French, 1988). However, these animals are able to restore the euthermic state in a very short period of time during the arousal process. Therefore, hibernating animals represent a suitable model to investigate, under physiological conditions, the effects of drastic changes in cell activity on cellular structure. In previous studies we demonstrated in several tissues of the hazel dormouse *Muscardinus avellanarius*, a wild-living true hibernator of small size, that during hibernation cell nuclei contain structural constituents absent in euthermia (Zancanaro et al., 1993; Malatesta et al., 1994a,b, 1995). In the present study we examine the same tissues in euthermic and hibernating individuals of the edible dormouse *Glis glis*, a true hibernator of larger size, in order to investigate possible modifications of nuclear structural constituents occurring during hibernation in this species.

MATERIALS AND METHODS

Seven adult individuals of the edible dormouse *Glis glis* were used in this study. Wild animals were trapped over 2 years in central Italy. This dormouse, like many other wild-living animals in Europe, is protected by law and only a limited number of individuals were available for the purpose of multiple investigations upon permission from local authorities. The animals were maintained in an external animal house and provided with food and bedding material; under such conditions they spontaneously began to hibernate in December and awoke in March. Four animals were sacrificed during hibernation (January, three after at least 6 days of continuous hibernation, one 36 hr after a periodic arousal) and three during the euthermic period (July). Dormant animals were taken from the cage and immediately killed by cervical dislocation. Euthermic animals were anaesthetised with ether and sacrificed as described above. Samples of liver, pancreas, brown adipose tissue (BAT) and adrenal cortex (cut in fragments encompassing the entire width of the gland) were quickly removed and small fragments were fixed by immersion in 4% paraformaldehyde in 0.1 M Sörensen phosphate buffer at 4°C for 2 hr. After washing in Sörensen buffer and in phosphate buffered saline (PBS), free aldehydes were blocked in 0.5 M NH_4Cl in PBS at 4°C for 45 min. Following washing in PBS, the specimens were dehydrated through graded concentrations of ethanol and embedded in LRWhite resin polymerized with UV light. Ultrathin sections were placed on grids coated with a Formvar-carbon layer and then processed according to the following protocols. In order to clearly identify the nuclear structural constituents containing ribonucleoproteins (RNPs), the sections were stained with the EDTA method (Bernhard, 1969). For immunocytochemical analyses the following antibodies were employed to characterize the structural components described in this study: a mouse monoclonal anti-(Sm)snRNP (small nuclear ribonucleoprotein) antibody (Lerner et al., 1981), a chicken antibody against the hnRNP (heterogeneous nuclear RNP) core protein (Jones et al., 1980), a rabbit polyclonal anti-coilin (Andrade et al., 1993) antibody, a mouse monoclonal antibody against the proliferation associated nuclear antigen (PANA) considered as a marker of interchromatin granules (IGs) (ICN Biomedicals, Clevenger and Epstein, 1984). Moreover, some other antibodies which did not show specific labelling on the structural constituents under analysis were tested: mouse monoclonal anti-(ds+ss)DNA antibody (Progen), rabbit polyclonal (kindly provided by Dr. G. Gabbiani) and mouse monoclonal (Sigma) anti-actin antibodies and rat monoclonal anti-tubulin (Sera-Lab) antibody.

Sections, placed on nickel grids, were floated for 3 min on normal goat serum (NGS) diluted 1:100 in PBS, and then incubated for 17 hr at 4°C with the primary antibody diluted with a solution containing 0.1% bovine serum albumin (BSA) (Fluka) and 0.05% Tween 20 in PBS. After rinsing, sections were floated on NGS, and then reacted for 30 min at room temperature with the secondary gold-conjugated antibody (Aurion) diluted 1:3 in PBS. When antibodies were raised in chickens, a secondary rabbit anti-chicken IgG (EY Labs; 1:100 in PBS-Tween-BSA) was applied for 30 min at room temperature prior to the gold-conjugated marker. Following the last incubation, all sections were rinsed, air-dried and, finally, stained with the EDTA method (Bernhard, 1969). As controls, some grids were treated with the incubation mixture without the primary antibody, and then processed as described above. In the case of the chicken antibodies, some grids were also incubated in the absence of both the primary antibody and the rabbit anti-chicken IgG bridge probe.

The specimens were observed in a Zeiss EM 902 and a Philips CM12 electron microscope operating at 80 kV.

RESULTS

Electron microscopic examination of hepatocytes, pancreatic acinar cells, brown adipocytes and adrenocortical cells (from the zona glomerulosa, fasciculata and reticularis) demonstrated that no significant differences in the basic ultrastructural features of cell nuclei occur between euthermic and hibernating dormice. The cell nuclei contained all the structural components generally observed in mammalian cell nuclei, regardless of the physiological state (Fig. 1A): perichromatin fibrils (PF) and perichromatin granules (PG) were abundant along the border of the condensed chromatin, while clusters of interchromatin granules (IG) were distributed in the nucleoplasm. The nucleoli exhibited well recognizable fibrillar centers, dense fibrillar and granular components; however, nucleoli in hibernating animals appeared less regular in shape and less compact than in euthermic individuals because of nucleoplasmic invaginations.

In addition to the usual structural components, the cell nuclei of deeply hibernating dormice contained different types of nuclear bodies (Table 1) which were not identified either in euthermic dormice or in the hibernating animal sacrified 36 hr after a periodic arousal: amorphous bodies (ABs) (Fig. 1B) and fibro-granular material (FGM) (Fig. 1A) were present in all tissues examined, while coiled bodies (CBs) (Fig. 1A) were observed in liver, pancreas and BAT. In the latter tissue CBs were sometimes found to be in contact with short bundles of nucleoplasmic fibrils (NFs) (Fig. 1C). In adrenal cortex and BAT we also found clustered granules of the size of PGs (PG-like granules) which, in brown adipocytes, were found frequently associated with bundles of NFs (Fig. 1D). No differences were found in the features of cell nuclei among adrenocortical cells from the three histological zones of the gland.

The FGM consisted in clumps of a fibro-granular network forming long trails meandering in the nucleoplasm; it was much more developed in hepatocyte nuclei than in brown adipocytes and adrenocortical cells, while in pancre-

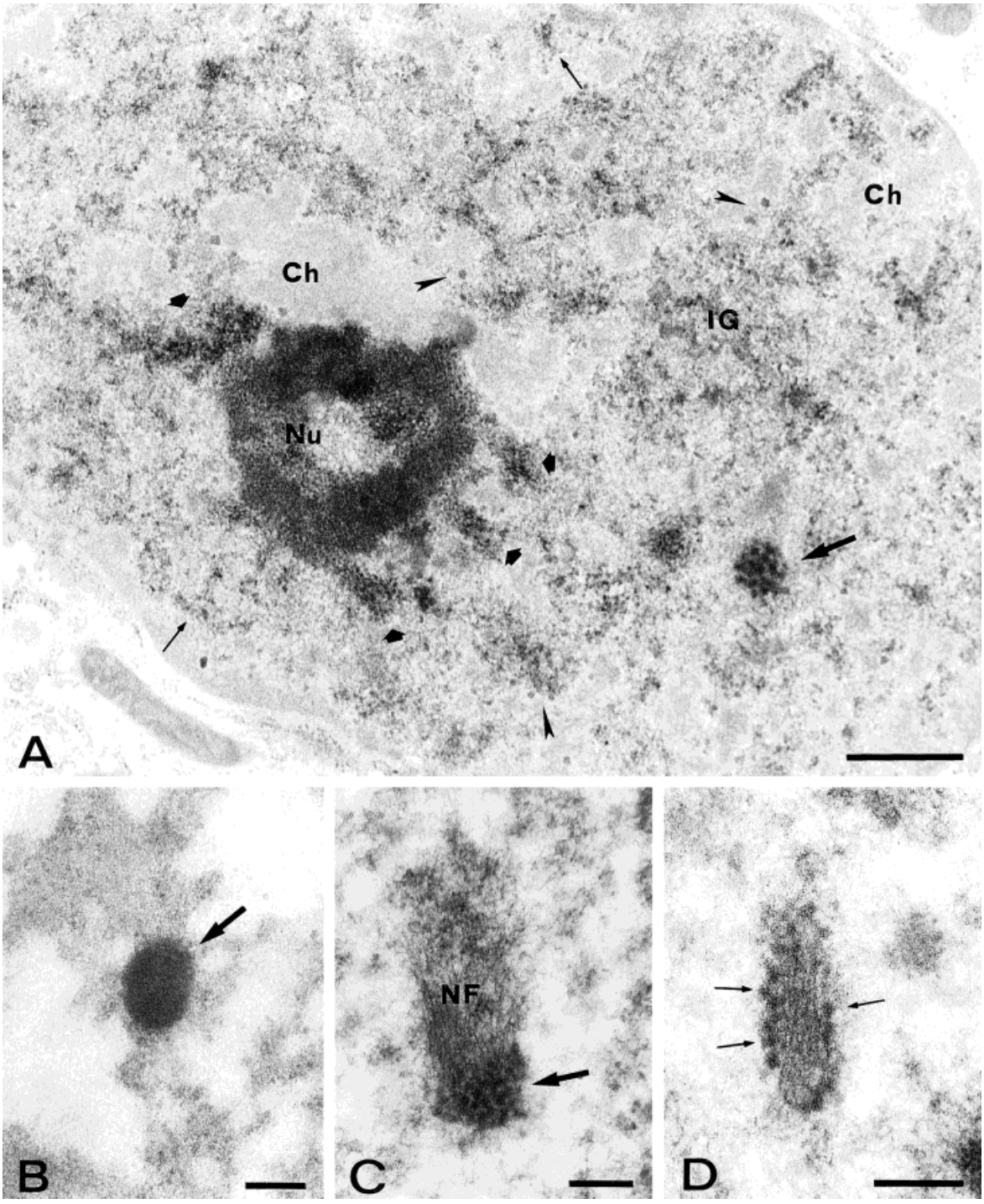

Fig. 1. Cell nuclei of hibernating edible dormice; EDTA staining. **A:** A general view of a hepatocyte nucleus showing abundant perichromatin fibrils (thin arrows) and perichromatin granules (arrowheads) along the border of the condensed chromatin (Ch), and clusters of interchromatin granules (IG). In addition, a coiled body (arrow) is present in the nucleoplasm and fibro-granular material (thick arrows) occurs in contact with the nucleolus (Nu). Scale bar = 0.5 µm. **B:** Adrenocortical cell nucleus showing an amorphous body (arrow). **C,D:** Brown adipocyte nuclei showing short bundles of nucleoplasmic fibrils associated with a coiled body (arrow) (C) and with granules lined up along the fibrils (thin arrows) (D). Scale bars = 0.2 µm.

atic acinar cells it was only occasionally observed. CBs exhibited the typical substructure consisting of tangled electron-dense threads, while ABs were poorly structured and strongly electron-dense, thereby resembling roundish, black spots. Such nuclear bodies were rather abundant in liver and pancreas (about 15% of sectioned nuclei showed one CB or AB), while in BAT their frequency was lower (about 4% of sectioned nuclei). NFs, occurring in brown

TABLE 1. Summary of the presence of the different nuclear constituents in the four tissues studied

	FGM	ABs	CBs	PG-like granules	NFs
Hepatocytes	x	x	x		
Pancreatic acinar cells	x	x	x		
Brown adipocytes	x	x	x	x	x
Adrenocortical cells	x	x		x	

adipocytes only, were constituted by thin fibrils associated to form short bundles which were frequently decorated with PG-like granules, which occurred lined up along the fibrils at the periphery of the bundle (Fig. 1D). On the other hand, in adrenocortical cell nuclei, where NFs were absent, PG-like granules occurred associated in clusters (not shown). All these nuclear constituents usually occurred in the nucleoplasm, apart from CBs and FGM, which were observed also in association with the nucleolus; in particular, CBs were always in intimate contact with the dense fibrillar component (Fig. 2A). All the nuclear bodies were strongly contrasted after EDTA staining. Immunocytochemical results (Table 2) revealed that FGM contain some snRNPs (Fig. 2D), hnRNPs (Fig. 3B) and PANA (Fig. 3D), ABs contain snRNPs (Fig. 2B) and hnRNPs (Fig. 3A), CBs contain snRNPs (Fig. 2A), coilin (Fig. 3C) and few hnRNPs (not shown), and PG-like granules contain some snRNPs (Fig. 2C). The anti-PANA antibody reacted relatively weakly with the edible dormouse antigen. NFs were not labelled with any of the probe used in this study. No difference in the immunolabelling of nuclear bodies was found among the different tissues. Moreover, no difference in labelling distribution on the usual structural components of the cell nucleus was observed between hibernating and euthermic animals. Control grids displayed negligible levels of labelling (not shown).

DISCUSSION

Our observations carried out on hepatocyte, brown adipocyte, pancreatic acinar cell and adrenocortical cell nuclei of hibernating and euthermic individuals of the edible dormouse *Glis glis* demonstrate the following points: 1) the cell nuclei of the tissues examined exhibit the same general features regardless of the physiological state of the animal; 2) fibro-granular material, coiled bodies, amorphous bodies, perichromatin granule-like granules and nucleoplasmic fibrils appear to be usual structural constituents of cell nuclei during deep hibernation, although their distribution seems peculiar to each tissue; 3) no one of these constituents is detectable in cell nuclei during euthermia.

The observations carried out on cell nuclei of the edible dormouse *Glis glis* are very similar to those previously reported on cell nuclei of the same tissues of the hazel dormouse (*Muscardinus avellanarius*) (Zancanaro et al., 1993; Malatesta et al., 1994a,b, 1995). In both hibernator species the general morphology of cell nuclei does not change significantly between euthermia and hibernation: this could be due to the continuation, although at a very low rate, of RNA and protein synthesis during the hibernating period (Bocharova et al., 1992; Derij and Shtark, 1985) and/or to the slowing down of molecular migration because of the low temperature, as previously observed in in vitro studies (Puvion et al., 1977). However, the main feature common to the two dormouse species is the presence of many particular nuclear bodies in the tissues of hibernating animals. In fact, all the nuclear bodies observed in edible dormice have been previously described—both morphologically and immunocytochemically—in the same tissues of hazel dormice. Since these structural constituents contain several splicing factors, we suggest (Malatesta et al., 1994a,b) that they may represent storage and/or assembly sites of such molecules which could be used for processing some RNA during hibernation (Bocharova et al., 1992; Derij and Shtark, 1985) and rapidly released upon arousal in order to meet the increased metabolic needs of the cell. In the particular case of CBs, it has been reported that their number increases in rapidly growing and metabolically activated cells (Lafarga et al., 1991; Spector et al., 1992; Andrade et al., 1993; Ochs et al., 1995). However, our findings are only apparently in disagreement with such observations; in fact, an accumulation of CBs could be necessary both in highly metabolising cells and in "quiescent" cells which must be able to quickly resume their activity at any time. As for NFs, the composition of which remains unknown, it could be hypothesised that they play some role in the dynamics of the associated nuclear structural components (CBs and PG-like granules), maybe facilitating their transport and utilisation upon arousal (Malatesta et al., 1995). The above hypotheses are supported by the observations carried out on the hibernating edible dormouse sacrificed 36 hr after a periodic arousal. In the tissues of this animal, in fact, none of the particular nuclear bodies described in this work was found, indicating that these structural constituents form during the hibernating bout and disappear when the euthermic functions are resumed.

Although nuclear bodies appear to be a common feature in tissues of hibernating edible and hazel dormice, there are some differences between the two species. Generally, the nuclear bodies are remarkably less frequent in edible dormice than in hazel dormice, especially in BAT and adrenal cortex. In particular, the adrenocortical cell nuclei of edible dormice contain only rare clusters of PG-like granules, a few ABs and some FGM and appear devoid of the nuclear bodies previously described in hazel dormice (see Malatesta et al., 1995). Moreover, in edible dormice some nuclear bodies have been found in tissues different from those of hazel dormice: for example the FGM occurs in large amounts in hepatocyte nuclei of the edible dormice, whereas in the same cells of the hazel dormice it was not observed; similarly, PG-like granules are present in BAT and adrenocortical cells of edible dormice, whereas in hazel dormice they were found in adrenocortical cells only. Finally, some nuclear bodies, although present in the two species, show some morphological differences: the ABs occurring in hepatocytes of edible dormice appear more regular in shape in comparison to those observed in the same tissue of hazel dormice, and the NF bundles observed in brown adipocytes of edible dormice are short and often associated with PG-like granules, whereas in hazel dormice the NFs widely meander in the nucleoplasm and are always devoid of granules (Malatesta et al., 1994a).

Although *Glis glis* and *Muscardinus avellanarius* are both Rodents classified in the same subfamily (Storch, 1994), the chromosomal analyses of the two species have revealed that they are genetically highly differentiated (Filippucci and Kotsakis, 1994; Zima et al., 1994). There-

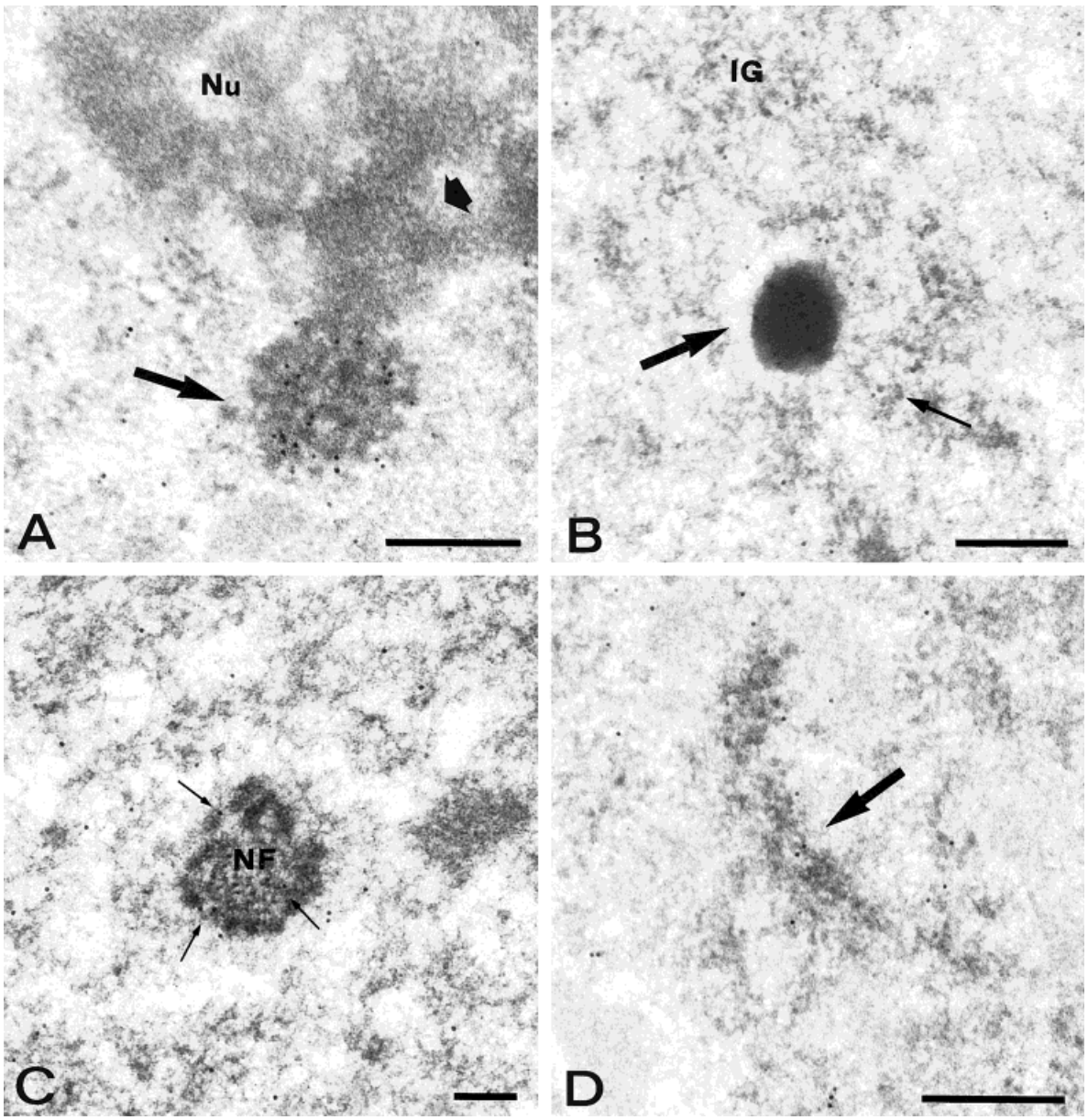

Fig. 2. Cell nuclei of hibernating edible dormice; immunolabelling with anti-snRNP (Sm) antibody; EDTA staining. **A:** Hepatocyte nucleus: a coiled body (arrow) showing specific labelling occurs in contact with the dense fibrillar component (thick arrow) of the nucleolus (Nu). **B:** Pancreatic acinar cell nucleus: the amorphous body (arrow) as well as perichromatin fibrils (thin arrow) and interchromatin granules (IG) are labelled. **C:** Brown adipocyte nucleus: the granules surrounding the bundle of nucleoplasmic fibrils show specific labelling (thin arrows). **D:** Hepatocyte nucleus: the fibro-granular material (arrow) contains some snRNPs. Scale bars = 0.2 µm.

fore, the differences observed could simply represent a consequence of the evolutionary differentiation. However, there could be a further explanation. It is known that no mammalian hibernator remains continuously at low body temperature all winter, but all hibernators rewarm and then briefly maintain high body temperatures at periodic intervals throughout the dormant season. Interestingly, the duration of these euthermic episodes depends on the animal size: large hibernators arouse slightly more frequently than do small ones and the duration of their euthermic intervals is much greater (French, 1985; Vogel, 1997). Therefore, the differences observed between the two

TABLE 2. Summary of the immunocytochemical results

	FGM	ABs	CBs	PG-like granules	NFs
Anti-snRNPs	+	2+	2+	±	−
Anti-hnRNPs	+	2+	±	−	−
Anti-PANA	±	−	−	−	−
Anti-p80-coilin	−	−	2+	−	−

−, not labelled; ±, weakly or occasionally labelled; +, labelled; 2+, strongly labelled.

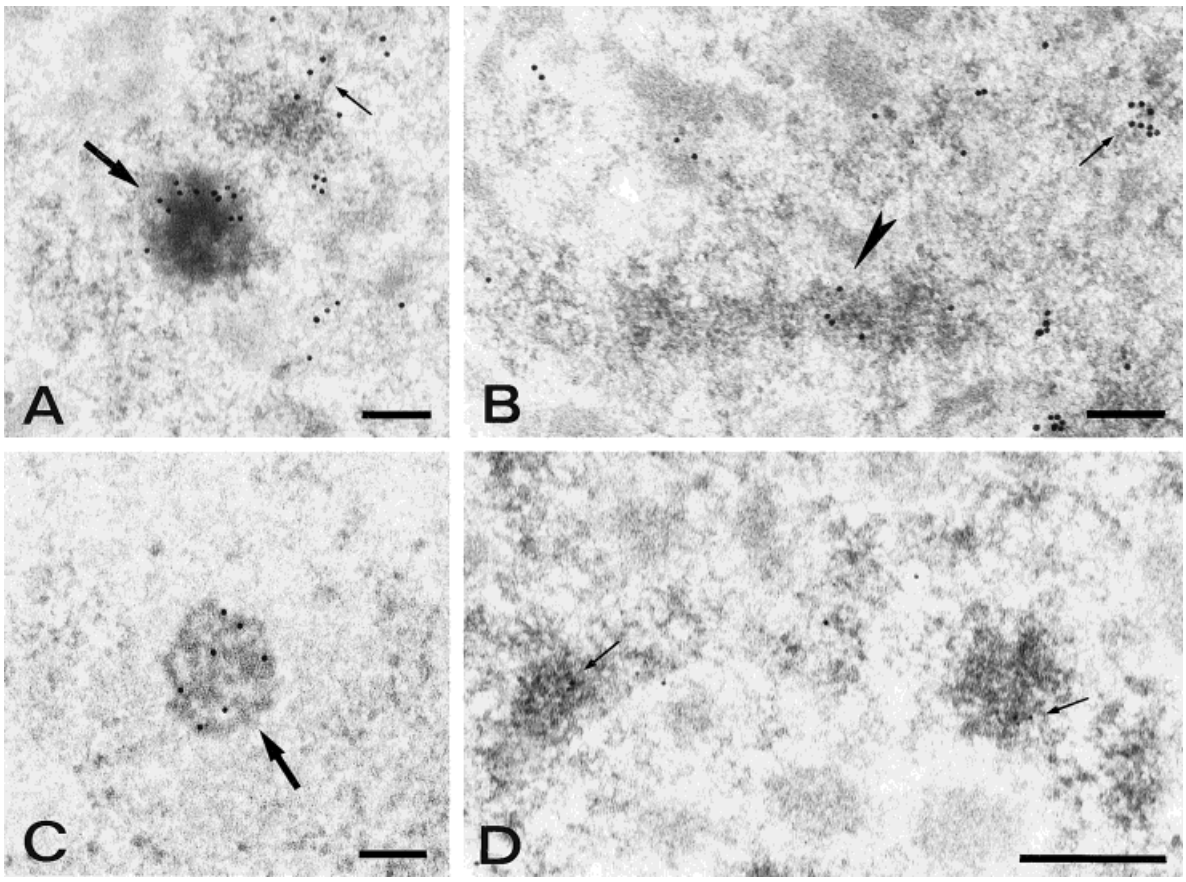

Fig. 3. Cell nuclei of hibernating edible dormice; EDTA staining. **A,B:** Hepatocyte nuclei immunolabelled with anti-hnRNP antibody: the amorphous body (arrow) and the perichromatin fibrils (thin arrow) are labelled, whereas the fibro-granular material (arrowhead) shows a weaker signal. **C:** Pancreatic acinar cell nucleus: the coiled body (arrow) is specifically labelled with the anti-coilin antibody. **D:** Adrenocortical cell nucleus: the fibro-granular material shows a weak but specific labelling (thin arrows) with the anti-PANA antibody. Scale bars = 0.2 µm.

species examined could be also correlated to the different characteristics of lethargy due to the remarkable difference in size between the edible dormouse (body weight of about 150–200 g) and the hazel dormouse (about 25–30 g in body weight). Interestingly, the main differences—quantitative as well as qualitative—about the nuclear bodies concern BAT and adrenal cortex. These tissues play key roles in the hibernator organism, especially upon arousal; in fact, the former is responsible for thermogenic activity (review in Himms-Hagen, 1986), and the latter is involved in control of hydro-electrolytic balance and metabolic processes (mainly protein and lipid catabolism for gluconeogenesis) (review in Nussdorfer, 1986).

In any case, the particular nuclear bodies observed only during deep hibernation seem to be strictly correlated to the hibernating state. If they are really involved in storage/assembly of splicing factors to be rapidly used upon arousal, these nuclear structural constituents could represent a usual feature in cells of hibernating species.

In order to elucidate this point, further studies are presently in progress on hibernating animal species phylogenetically distant from the dormice studied so far.

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