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Invited Review

The interchromatin granules

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Summary. In the present review, we report on the data obtained on the most prominent elements observed in the interchromatin spaces: the interchromatin granules (IGs). Special emphasis is placed on the recent contributions of immunocytochemical and in situ hybridization methods towards elucidating the composition and the behavior of these nuclear substructures during the cell cycle. The possible roles of IGs in the cell nucleus are also discussed.

Key words: Interchromatin granules, Nuclear organization, Cell cycle, Immunocytochemistry, Ultrastructure

Introduction

In the eucaryotic cell, cytoplasmic processes are organized within highly specialized membrane-bounded compartments such as mitochondria, lysosomes, the Golgi apparatus, and the endoplasmic reticulum. Although the nucleus has no membrane-bounded compartments, there is growing evidence that it is also organized into different functional domains (Spector, 1990). The best-characterized intranuclear domain is the nucleolus where rRNA genes are transcribed and pre-rRNAs are processed and assembled into preribosomal subunits (Scheer et al., 1993; Scheer and Weisenberger, 1994).

Except for the nucleolus, however, relatively little is known about the functional significance of various in situ structures described by morphologists. In the present article we briefly summarize the data obtained over the past 10 years on the most prominent elements observed in the interchromatin space: the interchromatin granules (IGs). Several reviews dealing with the fine structural morphology and cytochemistry of IG clusters have been previously published (Fakan and Puvion, 1980; Puvion and Moyne, 1981; Fakan, 1986). We therefore place special emphasis on the recent contributions of immunocytochemical and in situ hybridization methods towards elucidating the composition of these nuclear substructures. The possible functional roles played by IG in the cell nucleus are also discussed.

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Morphology

IGs are structural entities common to the nuclei of both normal and cancerous mammalian cells (Granboulan and Bernhard, 1961; Swift, 1963; Simard, 1966). Although similar structures have been observed in other eucaryotic, non-mammalian cells (Hügle et al., 1985; Medina et al., 1989; Jimenez-Garcia et al., 1992; Moreno Diaz de la Espina et al., 1992; Tremblay et al., 1992; Testillano et al., 1993), the homology of these structures with the IGs of mammalian cell nuclei remains to be clearly demonstrated.

IGs appear as irregular clusters of granules located in interchromatin spaces of nuclei (Bernhard and Granboulan, 1963, Fig. 1). The granules have a mean diameter of 200-250 angströms and with a frequently irregular shape. At higher magnification in ultrathin frozen sections, as in Epon sections, IGs appear to consist of fine, twisted fibrils. Many are also interconnected by fine fibrils producing a beaded chain appearance (Monneron and Bernhard, 1969; Kierszenbaum and Tres, 1974; Puvion and Bernhard, 1975).

Analyses of serial ultrathin sections indicate, on the other hand, that these IG clusters do not form a continuous network but rather occupy discrete sites of varying size within the nucleoplasm (Thiry, 1995b). Furthermore, the IGs form a part of the intranuclear ribonucleoproteins (RNP) network revealed after saline extraction of isolated nuclei (Shankar Narayan et al., 1967) or in thick frozen sections stained for RNP (Puvion and Bernhard, 1975).

Composition

DNA

IG clusters, it seems, are DNA-free. This view, initially based on the results obtained by various DNA-specific staining procedures (Cogliati and Gautier, 1973;

Moyne, 1973; Puvion and Bernhard, 1975; Zareba-Kowalska, 1989), is supported by more recent observations. Investigators using various specific anti-DNA antibodies (Turner and Franchi, 1987; Leser et al., 1989; Thiry, 1993; Visa et al., 1993a) and various in situ DNA detection methods (Turner and Franchi, 1987), such as the in situ terminal deoxynucleotidyl transferase-immunogold technique (Fig. 2), have failed to detect any DNA in IG.

However, small clumps of condensed chromatin and dispersed chromatin sensitive to DNase I have frequently been visualized at the borders and in the close vicinity of IG clusters (Thiry, 1993).

RNA

When subjected to RNP staining, IGs are the structures which prove most resistant to the chelating action of EDTA (Wassef, 1979). This high contrast cannot, however, be taken as unquestionable evidence that they contain RNA, since this technique stains RNP preferentially but not specifically. Surprisingly, apart from an autoradiographic study of rat liver cells treated with the adenosine analog DRB (Puvion et al., 1984b), studies have shown IG clusters to be rather scanty labeled after incubation for various periods with tritiated uridine (Fakan and Bernhard, 1971, 1973; Kierszenbaum, 1974; Fakan and Nobis, 1978). Moreover, although RNase T1- and RNAse T2-gold complexes do label these structures in the nuclei of untreated and DRB-treated cells (Cheniclet and Bendayan, 1990), RNAase A-gold fails to do so (Bendayan 1981 a,b). Recently, thanks to the use of various anti-RNA antibodies and especially to the

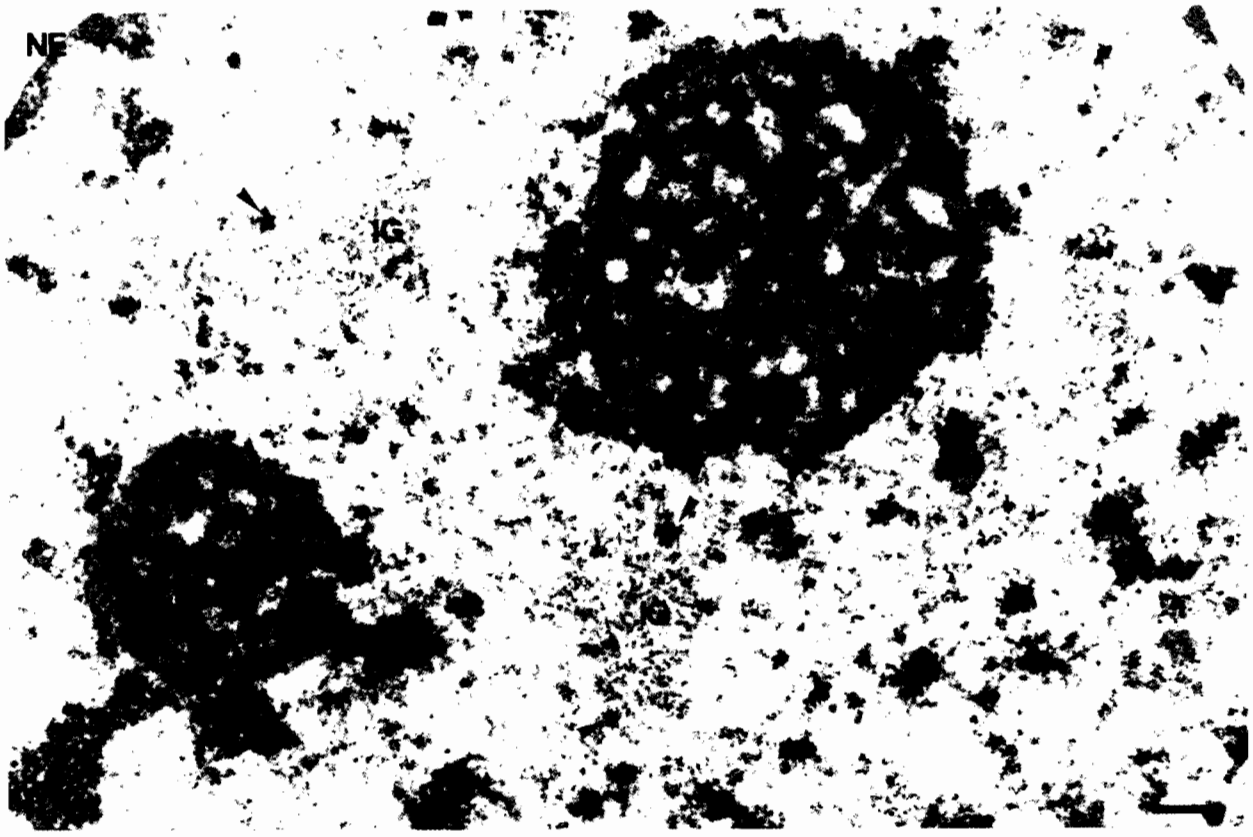


Fig. 1. Portion of a nucleus in a rat hepatocyte after acetylation. Besides the presence of the condensed chromatin (C) associated with the nuclear envelope (NE) and the two nucleoli (Nu), several interchromatin granule (IG) clusters are obvious in interchromatin spaces. These IG clusters are surrounded by small clumps of condensed chromatin (arrowheads). Bar= 1 μm.

Fig. 2. Distribution of DNA in a sample of rat hepatocytes after acetylation, as revealed by the terminal deoxynucleotidyl transferase-immunogold technique. Label is preferentially found over the blocks of condensed chromatin (C). Small clumps of condensed chromatin (arrows) surrounding the interchromatin granule (IG) cluster are also labeled. By contrast, the IG cluster is totally unlabeled. Bar= 1 μm.

Fig. 3. Distribution of RNA in a sample of HeLa cells after acetylation, as revealed by the polyadenylate nucleotidyl transferase-immunogold technique. Label is essentially found over the cytoplasm (T) and the interchromatin space (S). Inside the latter, a cluster of interchromatin granules (IG) and a coiled body (CB) are clearly labeled. By contrast, the condensed chromatin (C) is totally free of gold particles. Bar= 1 μm.

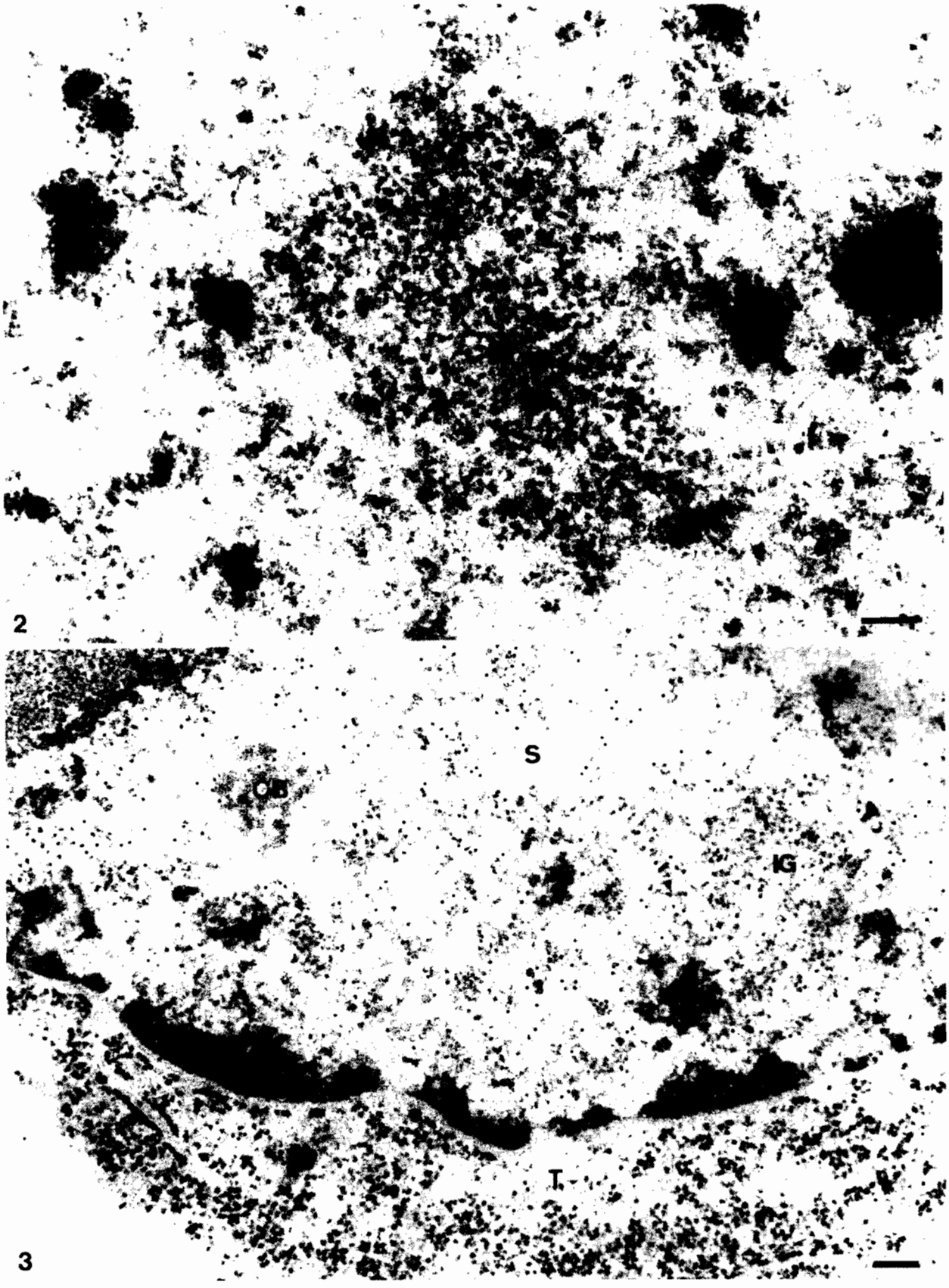

2
S
CB
IG
T
3

Table 1. *Proteins detected in the IG clusters by immunoelectron microscopy.*

ANTIGENS	MOLECULAR CHARACTERISITICS	REFERENCES
Sm	Proteins 29, 28, 16 and 13 KDa complexed with U1, U2, U4, U5 and U6 RNAs	Fakan et al., 1984; Puvion et al., 1984a; Smith et al., 1985; Leser et al., 1989; Spector et al., 1991; Raska et al., 1992; Visa et al., 1993a; Puvion-Dutilleul et al., 1994.
RNP	Proteins 33 and 22 KDa complexed with U1 RNA and protein 70 KDa associated with nuclear matrix	Puvion et al., 1984a; Leser et al., 1989
780-3	2 polypeptides of 105 and 41 KDa	Clevenger and Epstein, 1984a,b
p107	Protein 107 KDa associated with nuclear matrix	Smith et al., 1985
Ribocharin*	Protein 40 KDa specific to precursor particles of the large ribosomal subunit	Hügle et al., 1985
I14	Protein 40 KDa associated with nuclear matrix	Lehner et al., 1986
3C5	Proteins 130, 80, 50 and 34-50 KDa associated with nuclear matrix protein	Turner and Franchi, 1987; Raska et al., 1990; Ferreira et al., 1994
La/SSB	Phosphoprotein 48 KDa requires for termination of RNA synthesis by RNA polymerase III	Carmo-Fonseca et al., 1989
SC35	35 KDa non snRNP protein requires for spliceosome assembly	Spector et al., 1991; Raska et al., 1992
PAB II	Protein 49 KDa binds to the growing poly(A) tail, stimulating its extension	Krause et al., 1994

*: this antigen has been located in structures of amphibia resembling the IG clusters of mammalian cells, but homology with the latter has not been clearly established.

application of the in situ polyadenylate nucleotidyl transferase-immunogold technique (Fig. 3), a new and sensitive method for detecting RNA, it has been possible to demonstrate decisively the presence of an appreciable amount of RNA in IG clusters of untreated cells (Thiry, 1993, 1995a,b). Furthermore, IG clusters are labeled by antibodies raised against capped m3G (Reuter et al., 1985; Carmo-Fonseca et al., 1991b). The presence of UsnRNAs in IG clusters has been clearly confirmed by in situ hybridization (Carmo-Fonseca et al., 1991a,b, 1992; Huang and Spector, 1992; Matera and Ward, 1993; Visa et al., 1993a; Puvion-Dutilleul et al., 1994). The main substrate of splicing, poly(A) RNA, was also identified in IG clusters by hybridization at the ultrastructural level (Visa et al., 1993b; Huang et al., 1994; Puvion-Dutilleul et al., 1994). Under some experimental conditions, finally, such as in DRB-treated or adenovirus-infected cells, IG clusters have been found to contain rRNA (Puvion et al., 1984b) or viral RNA (Puvion-Dutilleul et al., 1992, 1994). In these particular cases, investigators have reported structural association between IG and the granular part of the nucleoli or the fibrillo-granular network of the viral compartment. The IG could thus be a meeting place of different kinds of RNA.

Proteins

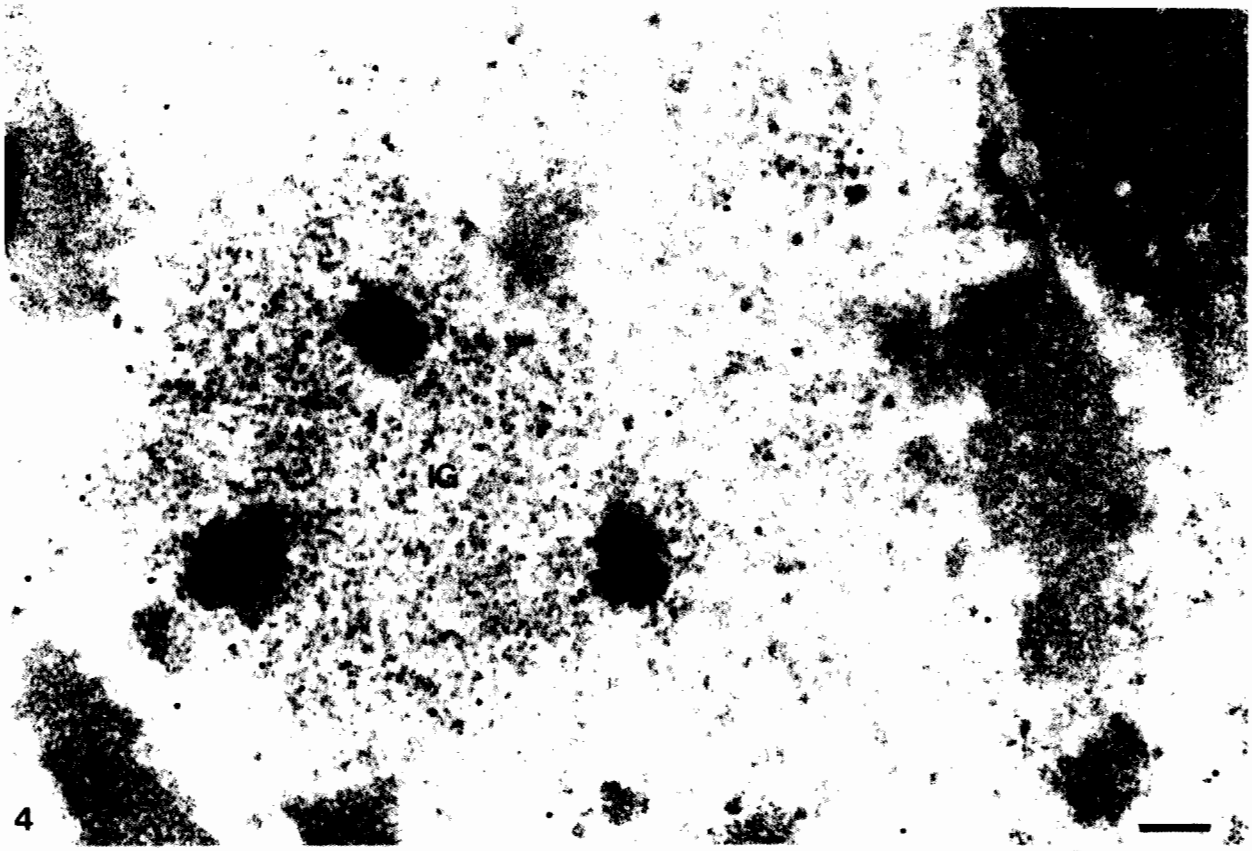
As summarized in Table 1, several proteins have been located inside the IG clusters by immunoelectron microscopy. In particular, IG clusters are comparatively rich in elements of the splicing machinery (Spector et al., 1983; Fakan et al., 1984; Puvion et al., 1984a; Spector et al., 1991; Carmo-Fonseca et al., 1992; Huang and Spector, 1992; Visa et al., 1993a; Figs. 4 and 5). Several proteins associated with nuclear matrix have also been predominantly identified in these structures (Smith et al., 1985; Turner and Franchi, 1987; Ferreira et al., 1994). Various cytochemical methods, notably bismuth staining, have revealed the presence of phosphoproteins in IGs (Brown and Locke, 1978; Wassef, 1979; Krzyzowska-Gruca et al., 1988). Moderate silver staining of IG clusters has been also observed (Dimova et al., 1982; Takeuchi and Takeuchi, 1984; Noaillac-Depeyre et al., 1989; Thiry, 1994, 1995b). The nature of the silver-stainable component of IG clusters, however, remains unknown.

IG during mitosis

Many authors have described the presence of mitotic structures morphologically similar to interphasic IG clusters (Fakan and Nobis, 1978; Clevenger and Epstein, 1984a,b; Takeuchi and Takeuchi, 1984; Turner and Franchi, 1987; Thiry, 1995b), but few studies (Leser et al., 1989; Thiry, 1995b) have examined the fate of IG at each step of mitosis. The behavior of IG during mitosis is schematically presented in the Figure 6.

Fig. 4. Electron micrograph showing the labeling of an ultrathin lowicryl section of actinomycin D-treated Ehrlich tumor cells with an anti-Sm serum from a patient suffering from systemic lupus erythematosus. Gold particles are particularly abundant over a cluster of interchromatin granules (IG) whereas the condensed chromatin (C) appears free of label. Bar= 1 µm.

Fig. 5. Electron micrograph showing the labeling of an ultrathin lowicryl section of a telophase HEp-2 cell with an anti-RNP serum from a patient suffering from mixed connective tissue disease. Gold particles are particularly abundant over a cluster of interchromatin granules (IG). Label is also found in the mitotic cytoplasm (T). The chromosomes (CH) are scanty labeled. Bar= 1 µm.



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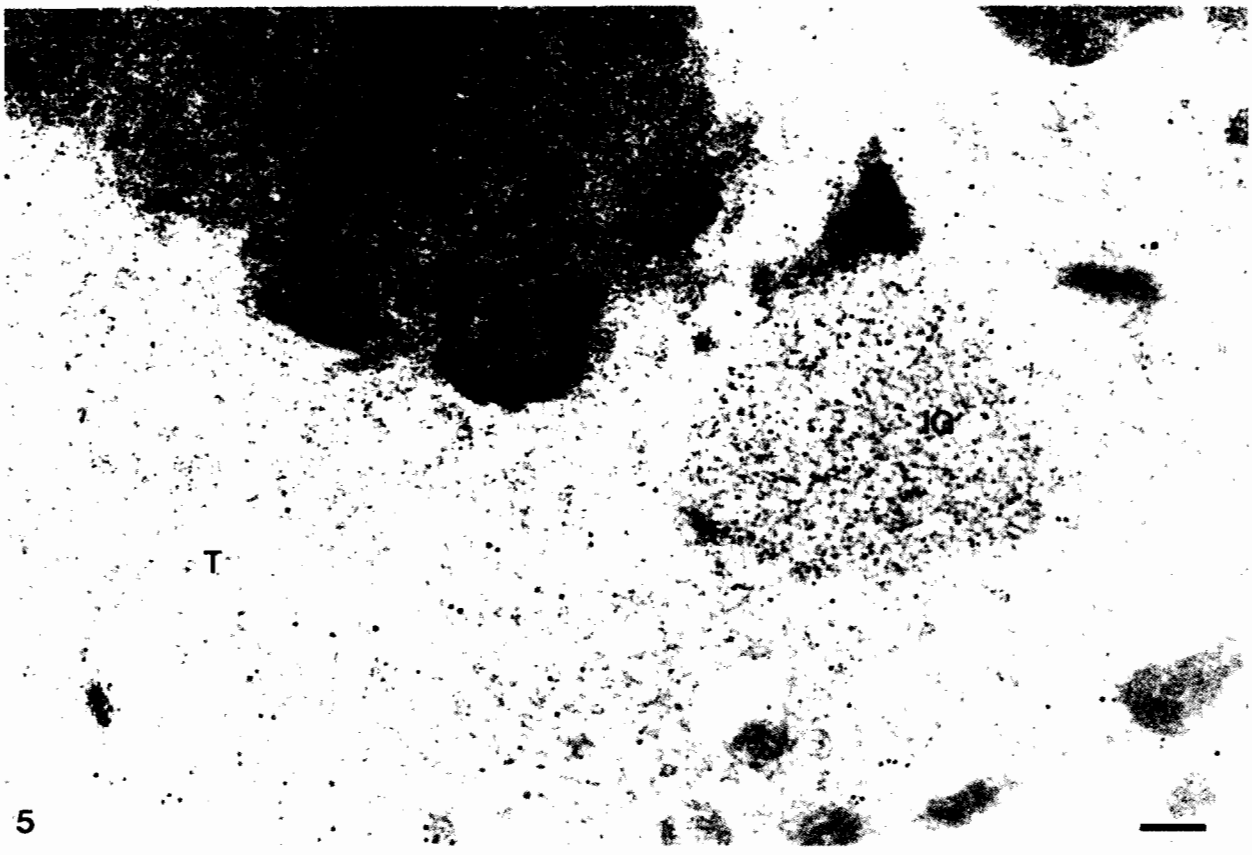

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During prophase (Fig. 7), the IGs aggregate in clusters which are more compact than in interphase cells. This phenomenon has also been described in many studies using various transcriptional inhibitors (Simard, 1966; Fedorko and Hirsch, 1969; Krzyzowska-Gruca et al., 1973; Sinclair and Brasch, 1978; Simard et al., 1979; Yu et al., 1983; Noaillac-Depeyre et al., 1989; Davis et al., 1993). At this stage, the surrounding clumps of condensed chromatin have disappeared. After breakdown of the nuclear envelope, these mitotic IG clusters disperse in the mitotic cytoplasm between and/or outside the chromosomes (Figs. 8, 9). Such granular structures have been reported frequently; they occur until the end of mitosis. During telophase, some of them come in close contact with the surface of re-forming nuclei. IG clusters reappear in the daughter nuclei at the level of these preferential contacts with the mitotic IG clusters. Concomitantly, the cytoplasmic IG clusters disappear.

On the other hand, the composition of interphase and mitotic IG clusters seem to be identical. Both appear to be DNA-free (Leser et al., 1989; Fig. 9). Both are stained by EDTA regressive staining (Fig. 8), preferential for RNPs, and are labeled by the polyadenylate nucleotidyl transferase-immunogold technique, specific for RNA-containing structures (Thiry, 1993, 1995b). Various protein antigens have been immunolocalized in interphase and mitotic IG structures (Clevenger and Epstein, 1984a,b; Turner and Franchi, 1987; Spector et al., 1991; Ferreira et al., 1994). High concentrations of snRNP antigens are notably detected (Leser et al., 1989; Fig. 5). No hnRNP proteins, on the other hand, are revealed in these structures (Leser et al., 1989), whose silver-stainability also persits throughout the cell cycle (Takeuchi and Takeuchi, 1984; Thiry, 1995b). One must remember, however, that the cytochemical and immunocytochemical methods used to obtain these data do not make it possible to appreciate quantitative variations. In the light of all these data, it clearly appears that the IGs, or some of them at least, persist throughout the cell cycle.

Functional significance

Considerable data support the view that IGs are involved in pre-mRNA metabolism. They do not, however, appear to be sites of mRNA synthesis in the nucleus, since they are not labeled when briefly pulsed with tritiated uridine (Fakan and Bernhard, 1971, 1973; Kierszenbaum, 1974; Fakan and Nobis, 1978), nor when treated with different anti-RNA-polymerase II antibodies (Spector et al., 1993), nor when subjected to various techniques for detecting DNA (Turner and Franchi, 1987; Leser et al., 1989; Thiry, 1993; Visa et al., 1993a). Another nucleoplasmic structural component, the perichromatin fibrils, is believed to consist of in situ nascent transcripts (Fakan, 1986, 1994). Three-

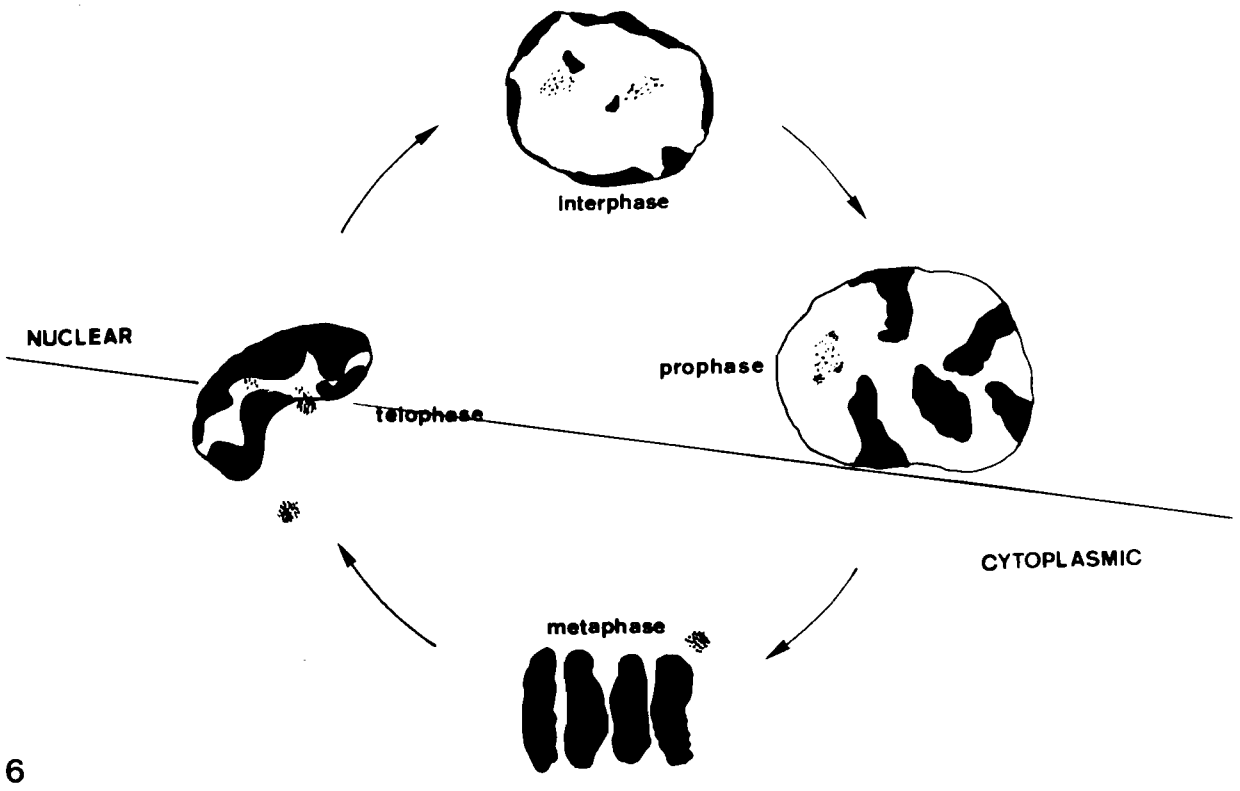

Fig. 6. Diagram illustrating the behavior of interchromatin granules during the cell cycle. Condensed chromatin is represented by black areas.

dimensional reconstruction at the fluorescence and electron microscope levels has shown some IG clusters to be interconnected by perichromatin fibrils (Spector, 1990). Interestingly, these preferential contacts decrease when cells are treated with an inhibitor of RNA polymerase II (Huang et al., 1994). IGs would thus appear to be involved in the posttranscriptional events of mRNA biogenesis.

It is notable, in this regard, that IG clusters are characterized by a high content in elements of splicing machinery (Spector et al., 1983; Fakan et al., 1984; Puvion et al., 1984a; Spector et al., 1991; Carmo-Fonseca et al., 1992; Huang and Spector, 1992; Visa et al., 1993a). Various snRNPs and non-snRNP splicing factors have been located in these structures. The main substrate of splicing, the poly(A) RNA, has also been detected in IG clusters (Visa et al., 1993b; Huang et al., 1994; Puvion-Dutilleul et al., 1994). On the other hand, a poly(A)-binding protein II, which strongly stimulates poly (A) tail extension, has been located preferentially in the IG clusters (Krause et al., 1994). Clearly, one must ask whether IGs are sites of pre-mRNA processing steps such as polyadenylation and splicing.

The tritiated uridine incorporation data, however, are not consistent with the view that poly(A) follows a pathway leading from perichromatin fibrils to IG clusters to the cytoplasm. In fact, only in cells pulse-labeled for 6 hours and chased for 24 hours was high-frequency labeling of the IG clusters observed (Fakan and Bernhard, 1973). In experiments detecting endogenous cfos RNA in serum-induced mouse fibroblasts, Huang and Spector (1992) further found that occurs, rather, at the periphery of speckles corresponding to IG clusters. Finally, at least some introns are removed cotranscriptionally (Beyer and Osheim, 1988) and an early association of UsnRNPs with extranucleolar RNA transcripts has been reported (Fakan et al., 1986). Thus, IG might be storage and/or reassembly sites for elements involved in mRNA polyadenylation and splicing (Huang and Spector, 1991; Jimenez-Garcia and Spector, 1993; Visa et al., 1993b). In this respect, it is interesting that IG clusters are frequently visualized in contact with different nuclear bodies (Visa et al., 1993a; Thiry, 1995b), notably the coiled bodies, another structural domain of the cell nucleus that has been implicated in pre- and post-splicing activities such as preassembly of snRNPs, degradation of introns, and recycling of snRNPs from post-splicing complexes (Lamond and Carmo-Fonseca, 1993). All these observations strongly favor the idea that perichromatin fibrils, the in situ form

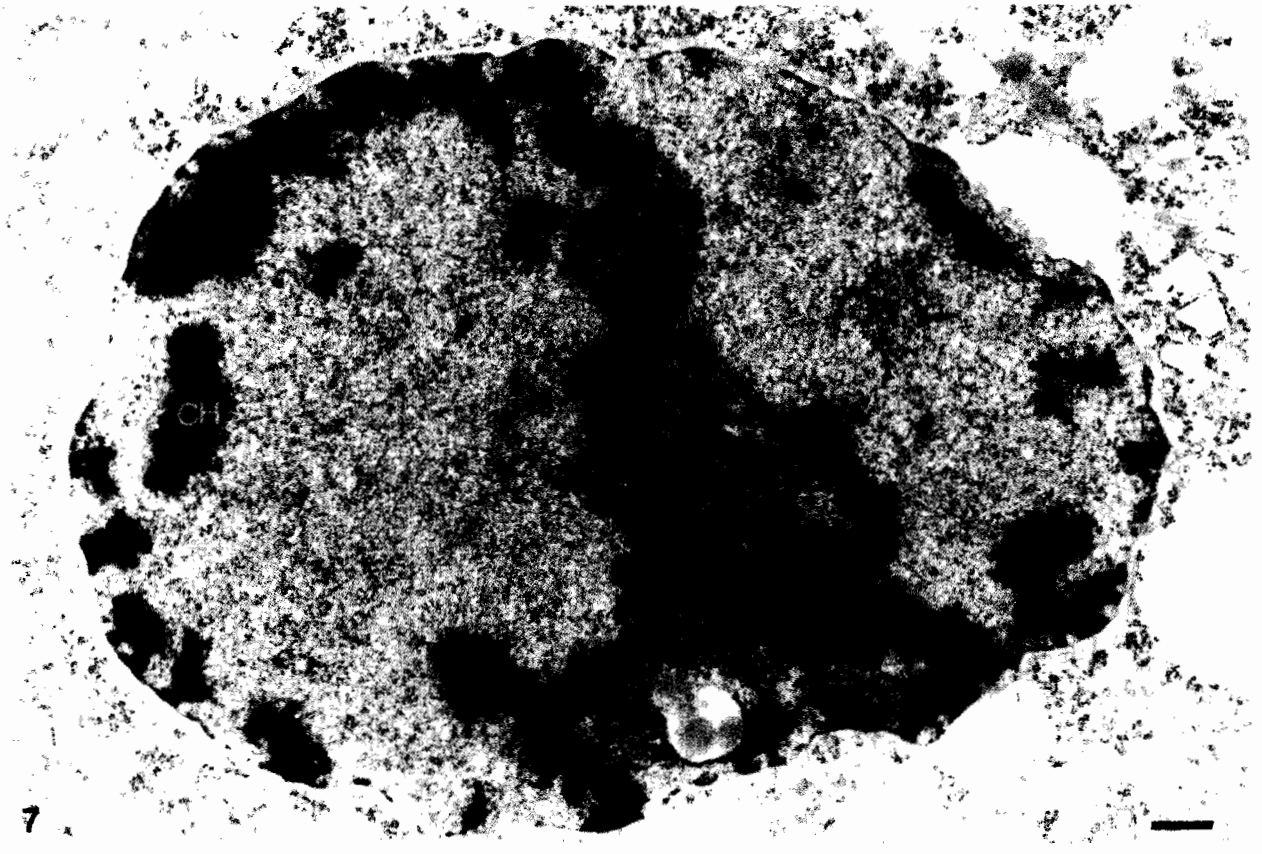

Fig. 7. Portion of a prophase Ehrlich tumour cell after acetylation. In the nucleus, the chromosomes (CH) individualize. Nucleolar material (Nu) is found between some condensing chromosomes. In the nucleoplasmic spaces, several interchromatin granule (IG) clusters are also quite obvious. Each is characterized by the presence of numerous peripheral agglomerates of granules (arrows). Bar= 1 μm.

of hnRNA transcripts, are also major sites of splicing and polyadenylation (Fakan, 1994). The poly(A) RNAs found in IG clusters are thus not pre-mRNAs but instead might constitute a stable population of mRNA involved in other nuclear functions (Huang et al., 1994). In agreement with this view, a significant fraction of nuclear poly(A) RNAs have been found to consist of sequences not detected in the cytoplasm (Herman et al., 1976). Examples of such poly(A) RNAs that remain in the cell nucleus have recently been reported (Brockdorff et al., 1992; Brown et al., 1992; Hogan et al., 1994), but their role in nuclear function is still unclear.

Several observations thus suggest that IGs could have a more general role in the cell nucleus. In fact, Puvion et al. (1984b) have shown by high-resolution autoradiography of in DRB-treated hepatocytes that rRNA was present in IG clusters. In Ad5-infected HeLa cells, likewise, viral RNAs were visualized in IG clusters by electron microscopy in situ hybridization (Puvion-Dutilleul et al., 1992, 1994). In both cases, contacts occurred between the IG clusters and the granular component of the nucleolus or the fibrillo-granular network of the viral compartment. The La protein, a factor required for termination of RNA polymerase III transcripts, has also been detected, by immunoelectron microscopy, in IG clusters (Carmo-Fonseca et al., 1989). The IG might thus play a role in the metabolism of various different nuclear RNAs.

IG may rightly be qualified as permanent structures of the cell. In addition to remnants of nucleoli, IGs constitute the only clearly recognizable in situ structural constituents of nuclear matrix preparations (Berezney, 1979). Several nuclear matrix proteins have been clearly identified in IG clusters (Smith et al., 1985; Turner and Franchi, 1987; Ferreira et al., 1994). Although IG reorganisation takes place during mitosis, IGs do not disappear and even seem to be transmitted, at least partly, from one generation to the next (Leser et al., 1989; Thiry, 1995b). Contrary to other nucleoplasmic structures of interphase cell nuclei, IG also persist in a wide variety of pathological situations resulting from treatment with various drugs or physical agents acting at

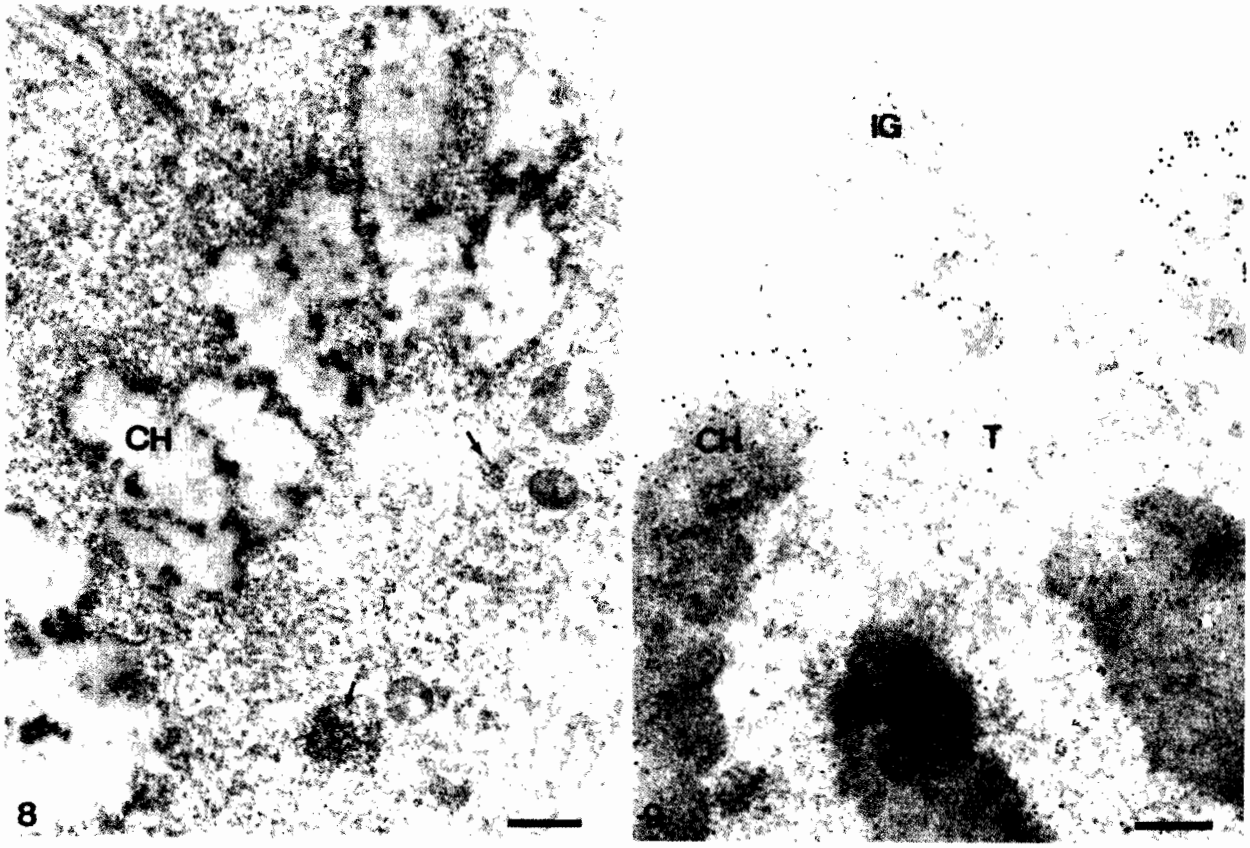

Fig. 8. Portion of an anaphase Ehrlich tumour cell after EDTA regressive staining preferential for RNP-containing structures. Whereas the chromosomes (CH) are completely bleached, the dense interchromatin granule (IG) clusters (arrows) dispersed in the mitotic cytoplasm exhibit high contrast. Bar= 1 µm.

Fig. 9. Portion of an Ehrlich tumour cell during metaphase after application of the in situ terminal deoxynucleotidyl transferase-immunogold technique for detecting DNA. Label is evident over the chromosomes (CH). By contrast, no label occurs over a dense interchromatin granule (IG) cluster. T: cytoplasm. Bar= 1 µm.

different metabolic levels (Simard et al., 1979).

Finally, it is also pertinent that all molecules detected to date in IG clusters, both proteins and RNAs, are also found in other nucleoplasmic structural constituents. On the basis of all these data, we propose that IG could play a central role in the cell nucleus, intervening in the organization and regulation of many nuclear RNAs.

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