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Relationship Between Nuclear Ribonucleoprotein Arrangement and Cell Proliferation in Burkitt Lymphoma Cells

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ABSTRACT. **The presence of body-like structures in nuclei from interferon alpha-treated Daudi cells has been shown on the ultrastructural level, by the use of different staining methods. The degree of their rearrangement in the nucleoplasm seems to be dependent on the time of interferon treatment. Since this morphological evidence has been found to be preceded by a slowing down of RNA transcriptional machinery early upon the interferon administration, it is speculated that interferon generated signals might lead to RNP granule accumulation in the nucleus and a consequent arrangement into defined structures.**

In recent years the mechanisms related to the transcriptional events such as processing and packaging of RNAs in the cell nucleus have been largely investigated, but so far, not completely clarified (16, 34, 32, 28). A number of investigations have shown that nascent RNA chains are associated with, and compacted by proteins which can produce a regular arrangement of particles of different sizes or be distributed irregularly but non-randomly (27, 11, 9, 1). These RNP particles have been demonstrated to be involved in a tremendous variety of cellular processes including protein targeting and transcription termination (19, 30, 13). Biological steps which include synthesis, assembly and maturation of transcripts, are followed by the release of RNAs from the nucleus for consequent metabolic events (20, 35, 31, 22). A number of agents like hormones, chemicals, growth factors and cytokines have been reported to influence cell metabolism by modulating the RNA transcriptional mechanisms. Among these agents, interferons have been largely evidenced to produce a wide spectrum of effects including modulation of gene expression, DNA metabolism and induction of antiviral state in different cell lines (12, 15, 23, 25, 7).

In this paper, we report an ultrastructural analysis, using different staining techniques, of RNP particle arrangement induced by interferon alpha in Burkitt lymphoma cells.

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MATERIALS AND METHODS

Cell culture and interferon treatment. Daudi cells were grown in stationary suspension culture at densities of 5×10^5 /ml in RPMI 1640 medium supplemented with 10% foetal calf serum, glutamine, penicillin-streptomycin and neomycin. Viability was determined by the trypan blue exclusion test. For experiments cells were treated with 500 I.U./ml of recombinant interferon alpha type A (Hoffmann-La Roche) for periods up to 72 hours.

Isolation of nuclei. Cells were resuspended in a buffer containing 2 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.4, 0.1 mM PMSF and 0.6% Triton X-100. The suspension was passed four times through a syringe, then $MgCl_2$ concentration was brought to 5 mM and nuclei recovered by low-speed centrifugation (i.e. $600 \times g$ for 10 min).

In vitro RNA synthesis. For the kinetic analysis of in vitro RNA synthesis, incubations were performed at 25°C for indicated times in a final volume of 300 μl containing 25 mM Tris-HCl, pH 8.0, 150 mM KCl, 1.5 mM dithiothreitol, 5 mM $Mg(Ac)_2$, 1 mM each ATP, CTP and GTP, 0.1 mM [^{3}H]UTP (175 cpm/pmol) and 15×10^6 nuclei. Aliquots of the reaction mixture were spotted, at the indicated times, on glass fiber filters (Whatman GF/C) and immersed in ice cold 5% TCA and 1% sodium pyrophosphate for a minimum of 10 min (21). Filters were washed in 1 N HCl and in ethanol, then air dried and counted in a nonaqueous scintillation fluid. The assay of RNA polymerase I activity was performed in the control and in 90-min treated nuclei in the presence of alpha amanitin (200 μg/ml) (29).

Silver staining. Daudi cells were washed in PBS and fixed in 1.6% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 for

15 min at 4°C. Cells were postfixed in Carnoy's solution (1 : 3 acetic acid : ethanol) for 4 min at 4°C. Cells were gradually rehydrated in ethanol and rinsed in distilled water for 10 min. The Ag-Nor procedure (14, 4) was applied. Cells were incubated for 10 min with 50% silver nitrate solution (1 g/2 ml H_2O) under an infrared light (G.E. Industrial 250 W) at a distance of 50 cm in order to reach 45 to 55°C. After a quick rinse in distilled water, the cells were incubated with an equal mixture (1 : 1) of ammoniacal silver solution and developing solution (3% formalin neutralized with sodium acetate and then adjusted to pH 5.3 with formic acid) for 1–2 min until the cells turned a pale yellow colour. Cells were rapidly rinsed with distilled water, dehydrated with increasing concentrations of ethanol, embedded in Epon 812 and sectioned for electron microscopy.

Bismuth staining. Cells were fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 1 h at 4°C. After 30-min washings in cacodylate and TEA HCl buffers, cells were stained with bismuth tartrate according to the literature (18). Bismuth solution was prepared as follows: 200 mg of bismuth oxynitrate was dissolved in 10 ml of 1 N NaOH containing 400 mg of sodium tartrate. When bismuth was completely dissolved, 20 ml of 0.2 M TEA HCl, pH 7.0, was added and the pH was readjusted to 7.0. Bismuth-stained cells were washed in 0.1 M TEA HCl buffer, pH 7.0, centrifuged to a loose pellet, ethanol-dehydrated and embedded in Epon 812. Thin sections were observed using an electron microscope withoug additional staining.

Bernhard's EDTA regressive staining. For Bernhard's staining fixation was carried out for 1 hour at 4°C in 2.5% glutaraldehyde in Sorensen's phosphate buffer, pH 7.3. After alcohol dehydration, the cells were embedded in Epon 812 according to the usual procedure. Sections were cut with an ultramicrotome equipped with a diamond knife, mounted on copper grids and stained according to the literature (3).

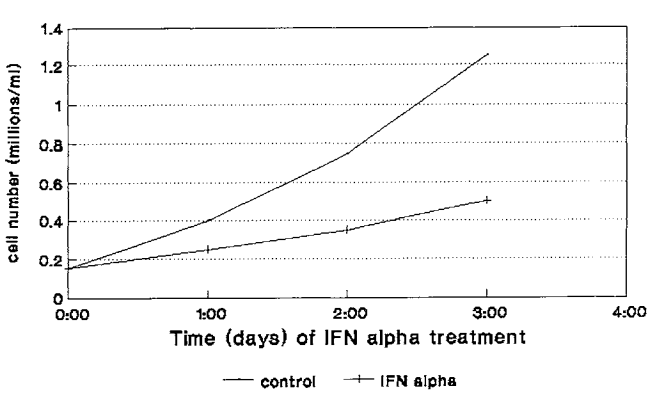

Fig. 1. Effect of recombinant interferon alpha type A on Daudi cell growth.

RESULTS

The effect of DNA human recombinant interferon alpha on Daudi cell growth rate is reported in Fig. 1. Cells

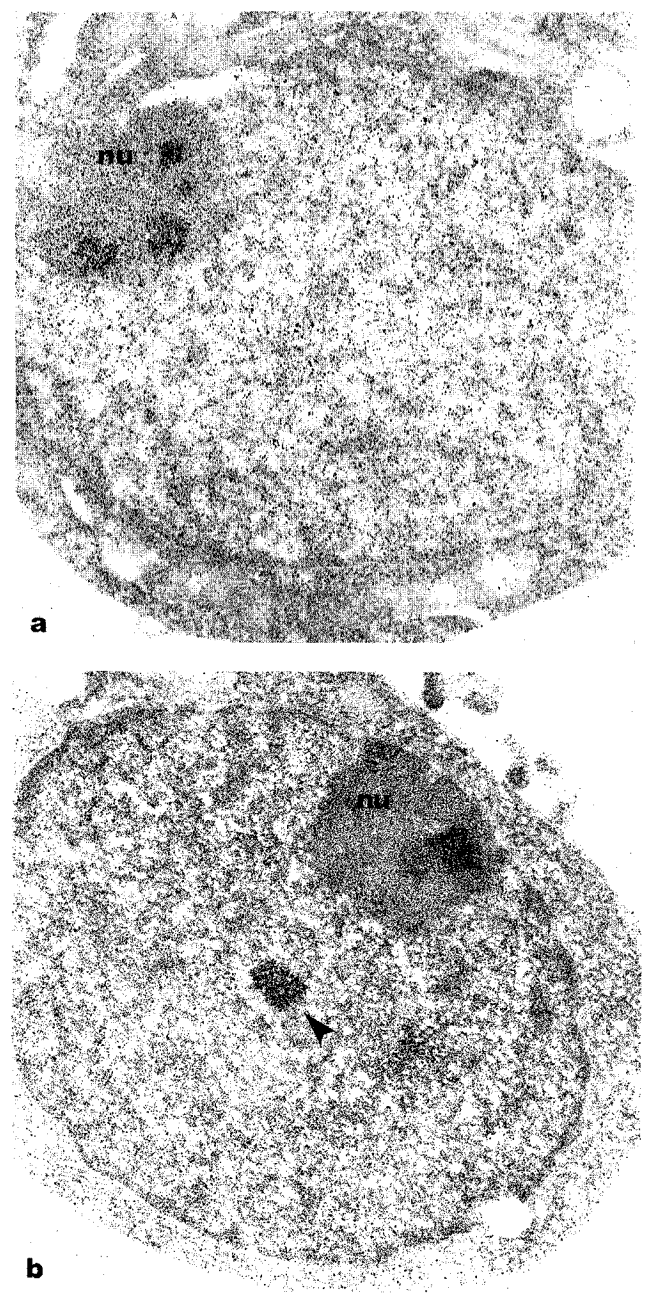

Fig. 3. Ultrathin sections of Daudi cells after silver staining. a=Control; b=after 72 hr interferon treatment. Arrowhead indicates a body-like structure. Nu: nucleolus. (×12,000).

Fig. 2. Ultrathin sections of Daudi cells after bismuth staining. Larger arrowheads indicate the body-like structures. a=Control; b=after 24 hr interferon treatment; c=after 48 hr interferon treatment; d=after 72 hr interferon treatment. Nu: nucleolus; ICG: interchromatin granules; FC: fibrillar center; DF: dense fibrillar component. ×12,000. Inset: high magnification (×40,000).

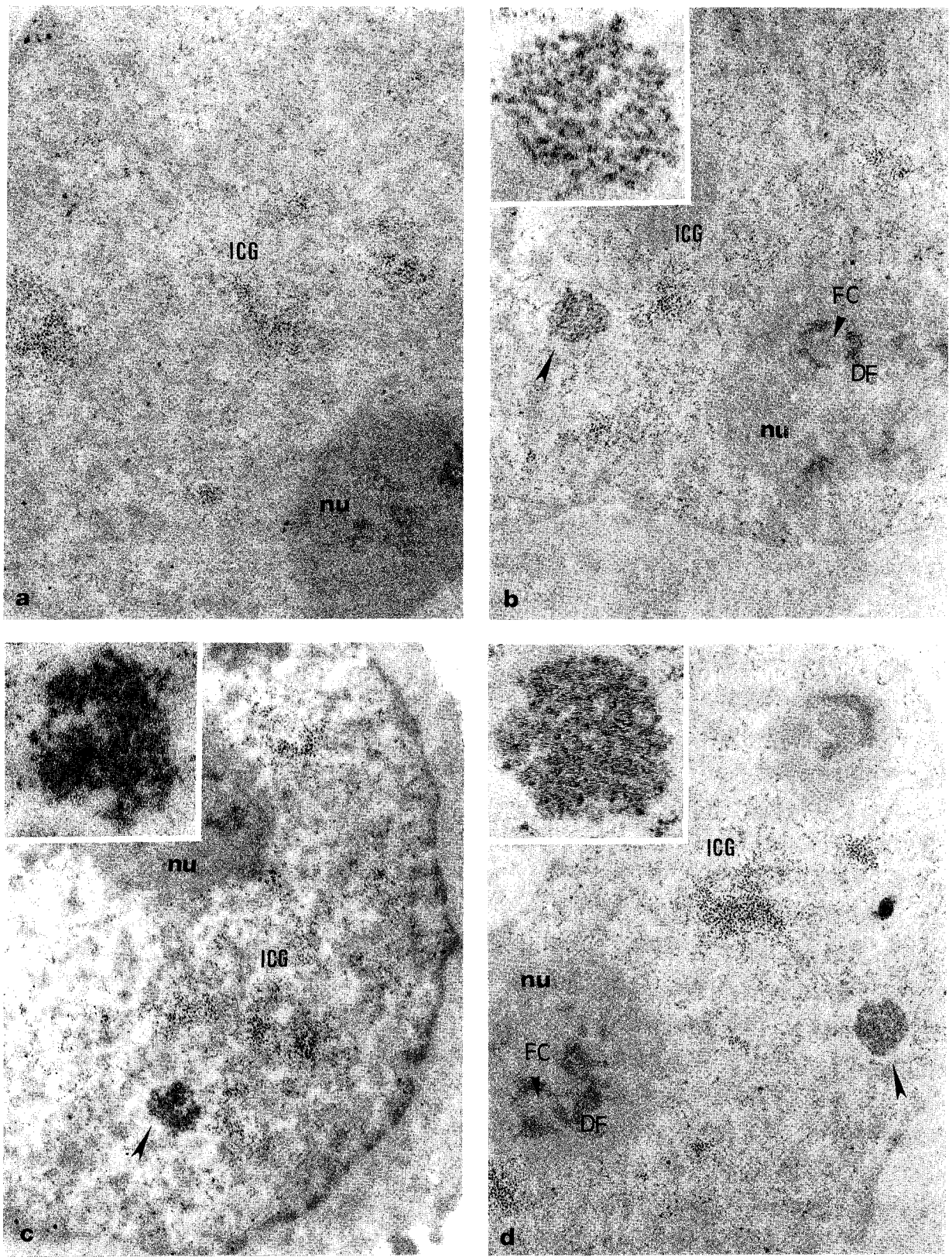

Fig. 2.

grown in unstirred suspension culture, double in number approximately every 30 hours. As shown, this cytokine progressively reduces cell growth within 36 hours and up to 3 days. Trypan blue exclusion test indicated that majority of the cells were still viable at least up to 3 days of interferon treatment. The ultrastructural analysis of control and treated cells has been performed by comparison of samples using the bismuth and silver staining techniques. In Fig. 2 ultrathin sections of control and interferon-treated samples after bismuth staining are shown. Sections evidence a progressive arrangement of perichromatin granules into body-like structures in treated samples with respect to the control. The occurrence of these body-like structures along with their condensation level increases with the time of interferon treatment (Table II and Fig. 2). In order to better characterize the nature of these body-like structure, silver staining and Bernhard's techniques were applied. Results (Fig. 3) disclosed that these structures can be detected also by silver staining due to their ribonucleoprotein nature. Furthermore, Bernhard's method has revealed that they were not bleached by EDTA suggesting that, most likely, they do not contain DNA (Fig. 4). It is

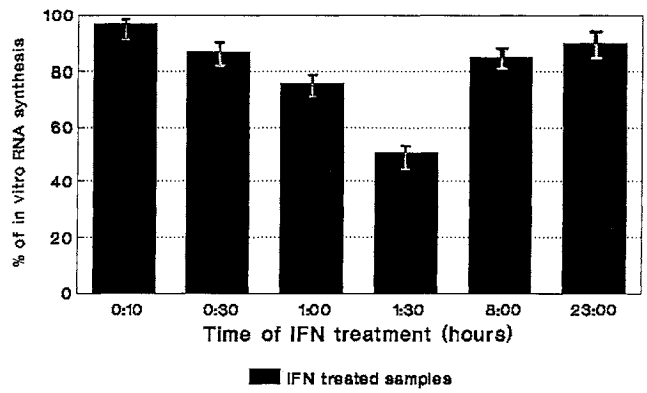

Fig. 5. In vitro endogenous RNA synthesis in nuclei isolated from interferon-treated Daudi cells. (100% refers to % of in vitro RNA synthesis of the control).
All data are the mean of at least five separate experiments.

worth noting that the occurrence of these morphological rearrangements is preceded by an early inhibition, with respect to the control, of in vitro total RNA synthesis in the isolated nuclei which is evident only up to 8 hr

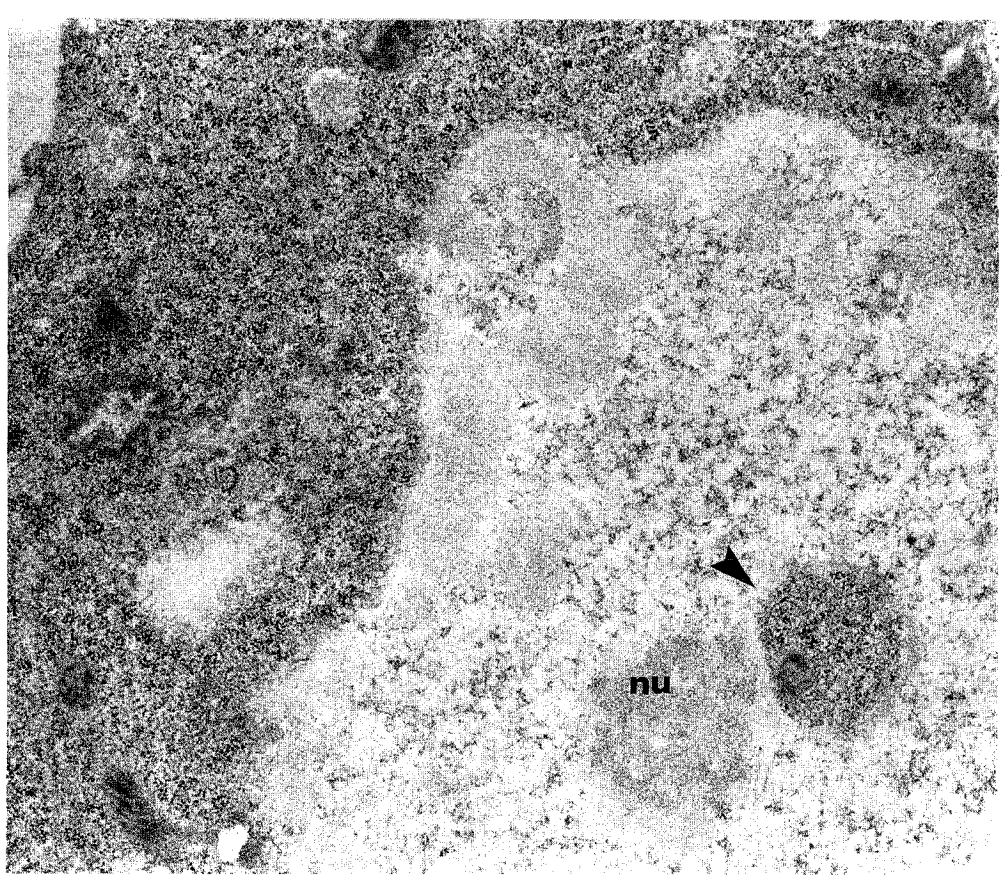

Fig. 4. Ultrathin section of a body-like structure (arrowhead) stained by Bernhard's method. (× 12,000).

Table I. KINETIC ASSAY OF RNA POLYMERASE I ACTIVITY IN NUCLEI ISOLATED FROM CONTROL AND INTERFERON-TREATED CELLS.

Spotting time	C	30'IFN	90'IFN	8 hr. IFN
0′	49±12	47±13	45±11	47±12
5′	291±32	232±30	162±29	285±35
20′	368±34	289±33	196±30	370±32
40′	406±38	296±31	211±28	412±36

Data are the mean of three separate experiments+S.D. C: Control

Table II. PERCENTAGE OF Daudi CELLS CONTAINING BODY-LIKE STRUCTURES.

Control	24 hr IFN	48 hr IFN	72 hr IFN
1	46	61	82

Cells counted per sample: 200.

of treatment. Fig. 5, in fact, shows a distinct reduction of 3H-UMP incorporation occurring early after interferon treatment which is progressively restored to control values within 8 hr. To further investigate the involvement of transcription process, the following were performed: upon interferon treatment, nuclei were incubated in the presence of alpha amanitin at a concentration of 200 μg/ml, which has been reported to inhibit RNA polymerases II and III. This allows us to monitor the effect of interferon on RNA polymerase I activity, which is responsible for rRNA synthesis. Table I shows with respect to the control, a reduction of the enzyme activity which is maximal at/or around 90 min of interferon treatment and returns to the control level within 8 hr of treatment.

DISCUSSION

The complexity, variety and versatility of RNP particles have emerged over the past few years from a large number of reports which have stressed their involvement in each step of the pathway of gene expression in eukaryotic cells (10, 17, 26). Here we report an intriguing rearrangement of RNP polyparticles into clusters which, often, can be observed around the periphery of the nucleolus and whose complexity level seems to increase with the time of interferon treatment. The nature of these body-like structures has been investigated by the use of different staining methods such as bismuth staining which, upon glutaraldehyde fixation, does not stain chromatin, in the nucleus but strongly contrasts perichromatin and interchromatin granules.

Interestingly, silver grains in nuclei indicate the same distribution as that of bismuth stain accounting for the main ribonucleoprotein nature of these body-like structures. This finding is also supported by the results from Bernhard's method which, revealing the absence or very slight occurrence of EDTA bleaching, strongly rules out for the DNA content of these structures. It is likely that bismuth and silver revealed granules derived from part of the RNA complexed into fibrils folded into a structure visualized as that of perichromatin granules which must be transported to the cytoplasm for subsequent protein processing (22). The finding of such clusters, of which the main RNP composition has been determined, allows us to speculate on the idea of an impairment of transcriptional machinery by interferon-generated signals in the nucleus. Furthermore, the result of an early decrease of in vitro RNA transcription occurring upon interferon treatment, which may induce the accumulation of the RNP granules in the nucleus and, in turn, their arrangement into dense bodies during the time of interferon treatment is of much interest. Since the inhibition of interferon on cell proliferation becomes evident around 24 hr after treatment, the possibility of a cascade of early signals generated by the interactions of this cytokine with cell surface receptors (37, 24, 5, 6, 8) can be envisaged leading to the occurrence, at ultrastructural level, of such body-like structures. The possibility of association of these structures to the elsewhere reported nuclear dense bodies (2, 36, 33), whose presence in the nucleoplasm has been related to cell activity, remains to be investigated.

REFERENCES

1. AMERO, S.A., RAYCHAUDHURI, G., CASS, C.L., VAN VENROOIJ, W.J., HABETS, W.J., KRAINER, A.R., and BEYER, A.L. 1992. Independent deposition of heterogeneous nuclear ribonucleoproteins and small nuclear ribonucleoprotein particles at sites of transcription. *Proc. Natl. Acad. Sci. USA,* **89**: 8409.
2. BARLOW, P.W. 1983. Changes in the frequency of two types of nuclear body during the interphase of meristematic plant cells. *Protoplasma,* **118**: 104–113.
3. BERNHARD, W. 1969. A new staining procedure for electron microscopical cytology. *J. Ultrastruct. Res.,* **27**: 250–265.
4. BOURGEOIS, C.A., HERNANDEZ VERDUN, D., HUBERT, G., and BOUTEILLE, M. 1979. Silver staining of NORs in electron microscopy. *Exp. Cell Res.,* **123**: 449–452.
5. CATALDI, A., MISCIA, S., LISIO, R., RANA, R., and COCCO, L. 1990. Transient shift of diacylglycerol and inositol lipids induced by interferon in Daudi cells. *FEBS letters,* **269**: (2) 465–469.
6. CATALDI, A., RANA, R., DI PRIMIO, R., BAREGGI, R., LISIO, R., TOTO, N., and MISCIA, S. 1992. Interferon mediated intracellular signalling. Modulation of different phospholipase activities in Burkitt lymphoma cells. *FEBS letters,* **313**: (3) 210–213.
7. CATALDI, A., SANTAVENERE, E., VITALE, M., TRUBIANI, O., LISIO, R., TULIPANO, G., DI DOMENICANTONIO, L., ZAMAI, L., and MISCIA, S. 1992. Interferon affects cell growth progression by modulating DNA polymerases activity. *Cell Prolif.,* **25**: 225–231.
8. CATALDI, A., LISIO, R., RANA, R., BAREGGI, R., TULIPANO, G., and MISCIA, S. 1993. Interferon rapidly induces modifications

of chromatin sensitivity to DNase I in Daudi lymphoma cells. *Exp. Cell Res.*, **204**: 167–202.
9. Choi, Y.D., Garbowski, P.J., Sharp, P.A., and Dreyfuss, G. 1986. Heterogeneous nuclear ribonucleoproteins role in splicing. *Science*, (Wash. D.C.) **231**: 1534–1539.
10. Dreyfuss, G., Philipson, L., and Mattaj, I.W. 1988. Ribonucleoprotein particles in cellular processes. *J. Cell Biol.*, **106**: 1419–1425.
11. Fakan, S., Leser, G., and Matin, T.E. 1984. Immunoelectron microscope visualization of nuclear ribonucleoprotein antigens within spread transcription complexes. *J. Cell Biol.*, **103**: 1153–1157.
12. Friedman, R.M. 1981. *Interferons: a primer*. New York Acad. Press.
13. Gall, J.G. 1991. Spliceosomes and snurposomes. *Science*, **252**: 1499–1500.
14. Goodpasture, C. and Bloom, S.G. 1975. Visualization of nucleolar organizer regions in mammalian chromosomes using silver staining. *Chromosoma*, **53**: 37–50.
15. Grimley, P.M., Kang, J.H., Silvermann, R.H., Davis, G., and Hoognagle, J.H. 1983. Blood lymphocites inclusions associated with alpha interferons. *Lab. Invest.*, **48**: 30–31.
16. Hancock, R. and Boulikas, T. 1982. Functional organization in the nucleus. *Int. Rev. of Cytology*, **79**: 165–214.
17. Le Stourgeon, W.M., Barnett, S.F., and Northington, S.J. 1990. In *The eukaryotic nucleus* (P. Strauss and S. Wilson eds.). Telford, Caldwell, NJ **2**: 477–502.
18. Locke, M. and Huie, P. 1976. The beads in the Golgi complex endoplasmic reticulum region. *J. Cell Biol.*, **70**: 384–394.
19. Maniatis, T. and Reed, R. 1987. The role of small nuclear ribonucleoprotein particles in pre-mRNA splicing. *Nature (London)*, **325**: 673–678.
20. Manley, J. 1984. Transcription of eukaryotic genes in whole cell extracts in *Transcription and translation* **3**: 71–88.
21. Marzluff, W.F., Murphy, E.C., and Chih Huang, R. 1973. Transcription of ribonucleic acid in isolated mouse myeloma nuclei. *Biochemistry*, **12**: (18) 3440–3446.
22. Mehlin, H., Daneholt, B., and Skoghund, N. 1992. Translocation of specific premessenger ribonucleoprotein particle through the nuclear pore studied with electron microscope tomography. *Cell*, **69**: (4) 605.
23. Miscia, S., Cataldi, A., and Grimley, P.M. 1989. Influence of phosphatidylcholine on two protein mRNA levels in interferon treated Daudi cells. *Cell Biol. Int. Rep.*, **13**: (7) 595–600.
24. Miscia, S., Cataldi, A., and Grimley, P.M. 1990. Early modifications of nucleic acid metabolism and nuclear lipid biosynthesis associated with antiproliferative activity of interferon alpha on Daudi lymphoma cells. *Exp. Cell Res.*, **187**: 197–202.
25. Miscia, S., Cataldi, A., Lisio, R., Tulipano, G., Rizzoli, R., Rana, R., and Cocco, L. 1991. Interferon transiently modulates intranuclear signalling system in erythroleukemia friend cells. *Cell Biol. Int. Rep.*, **15**: (5) 427–435.
26. Myer, V.E., Lee, S.I., and Steitz, J.A. 1992. Viral small nuclear ribonucleoproteins bind a protein implicated in messenger RNA destabilization. *Proc. Natl. Acad. Sci. USA*, **89**: 1296–1300.
27. Paiement, J. and Bendayan, M. 1982. Localization of RNA in incubated rat liver nuclei. *J. Ultrastruct. Res.*, **81**: 145–157.
28. Rajpurohit, R., Paik, W.K., and Kim, S. 1992. Enzymatic methylation of heterogeneous nuclear ribonucleoprotein in isolated liver nuclei. *Biochim. Biophys. Acta*, **1122**: 183–188.
29. Roeder, R.G. 1976. In *RNA polymerase* (R. Losick and M. Chamberlain eds.). Cold Spring Harbor Lab. Press N.Y. 285.
30. Sachs, A.B., Davis, R.W., and Kornberg, R.D. 1987. A single domain of yeast poly (A) binding protein is necessary and sufficient for RNA binding and cell viability. *Mol. Cell. Biol.*, **7**: 3268–3276.
31. Siegel, V. and Walter, P. 1985. Elongation arrest is not a prerequisite for secretory protein translocation across the microsomal membrane. *J. Cell Biol.*, **100**: 1913–1921.
32. Swanson, M.S. and Dreyfuss, G. 1988. Classification and purification of proteins of heterogeneous nuclear ribonucleoprotein particles by RNA binding specificities. *Mol. Cell. Biol.*, **8**: 2237–2241.
33. Tremblay, S.D., Gugg, S., and Lafontaine, J.G. 1992. Ultrastructural, cytochemical and immunocytochemical investigation of the interphase nucleus in the unicellular green alga Chlamydomonas reinhardtii. *Biol. Cell*, **76**: 73–86.
34. Vazquez, Nin, G., Ortiz, A., Lopez, A., and Echeverria, O. 1989. Electron microscope study of ribonucleoprotein polyparticles and their relation to perichromatin granules. *Biology of the Cell*, **65**: 133–138.
35. Walter, P. and Blobel, G. 1983. Disassembly and reconstitution of signal recognition particle. *Cell*, **34**: 525–533.
36. Williams, L.M., Jordan, E.G., and Barlow, P.W. 1983. The ultrastructure of nuclear bodies in interphase plant cell nuclei. *Protoplasma*, **118**: 95–103.
37. Yap, W.H., Teo, T.S., McCoy, E., and Tan, Y.H. 1986. Rapid and transient in DAG concentration in Daudi cells exposed to interferon. *Proc. Natl. Acad. Sci. USA*, **83**: 7765–7769.

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