Journal of Cell Science 104, 853-859 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

Decrease in nuclear phospholipids associated with DNA replication

Nadir M. Maraldi[1,*], Spartaco Santi[2], Nicoletta Zini[1], Andrea Ognibene[2], Riccardo Rizzoli[3], Giovanni Mazzotti[3], Roberto Di Primio[4], Renato Bareggi[5], Valeria Bertagnolo[6], Carla Pagliarini[6] and Silvano Capitani[6]

[1]Istituto di Citomorfologia Normale e Patologica del C.N.R. c/o Istituto di Ricerca Codivilla-Putti, I.O.R., 1/10 Via di Barbiano, 40136 Bologna, Italy
[2]Laboratorio di Biologia Cellulare e Microscopia Elettronica, I.O.R., Bologna, Italy
[3]Istituto di Anatomia Umana Normale, Università di Bologna, Bologna, Italy
[4]Istituto di Morfologia Umana Normale, Università di Chieti, Chieti, Italy
[5]Istituto di Anatomia Umana Normale, Università di Trieste, Trieste, Italy
[6]Istituto di Anatomia Umana Normale, Università di Ferrara, Ferrara, Italy

*Author for correspondence

SUMMARY

Lipid metabolism in nuclei is very active and appears involved in the transduction of signals to the genome in response to agonists acting at the plasma membrane level. However, the precise topology of nuclear lipid metabolism and the relationship between nuclear lipids and crucial events of the cell function, such as DNA replication, have not been fully elucidated. By using a recently developed cytochemical method for detecting phospholipids inside the nucleus of intact cells at the electron microscope level, we have analyzed the changes in intranuclear phospholipids in DNA-replicating versus resting cells, which are both present in the same sample of regenerating liver after partial hepatectomy.

The pattern of DNA synthesis in replicating cells has been monitored by electron microscope immunocytochemistry after bromodeoxyuridine (BrdU) labeling. The data obtained, which allow a fine localization and a quantitative analysis of both DNA synthesis and phospholipid distribution, indicate a significant reduction in the phospholipids detectable inside the nucleus in all steps of the S phase. This could depend on an increased nuclear phospholipid hydrolysis, whose products should in turn activate some of the enzymes involved in the control of DNA replication.

Key words: immunocytochemistry, electron microscopy, nuclear phospholipids, S phase, bromodeoxyuridine

INTRODUCTION

One of the more-studied models for DNA replication, in *in vivo* conditions, is represented by liver regeneration after partial hepatectomy. The signals which elicit the proliferating response have been partly identified; hormones like insulin and vasopressin and growth factors such as epidermal growth factor (EGF) (Cruise et al., 1985, 1987; Olsen et al., 1988) play a role in this phenomenon, which involves, as a basic mechanism, protein phosphorylation mediated by protein kinases, both dependent on cyclic nucleotides (Laks et al., 1981) and activated by the inositol lipid cycle (Okamoto et al., 1988).

The recently demonstrated presence of protein kinase C (PKC) in isolated rat liver nuclei (Azhar et al., 1987; Capitani et al., 1987; Rogue et al., 1990) and the increased phosphorylation of exogenous histone H1 by PKC occurring after partial hepatectomy (Martelli et al., 1991a), as well as the demonstration that exogenous PKC is able to hyper-phosphorylate some nuclear proteins in regenerating liver (Mazzoni et al., 1992b), suggest that the onset of DNA replication might involve intranuclear PKC. The enzyme is regulated by lipids and lipid-generated cofactors, which can originate from inositol lipids and other phospholipids (Billah and Anthes, 1990). Inositol lipids, and other phospholipid species as well, have been detected by different methods in isolated nuclei (for a review see Maraldi et al., 1992a); therefore, the nucleus owns a sufficient set of factors capable of eliciting the first steps of DNA replication in response to a flow of lipid-dependent signals either generated at the cell surface or transduced to the cell interior via an unknown mechanism.

Regulation of PKC activity in the nucleus, which can be affected primarily by products of the inositol lipid cycle (Divecha et al., 1991; Martelli et al., 1992; Michell, 1992), could depend also on phosphatidylserine (PS), phosphatidylcholine (PC) and other lipids as assumed for cytoplasmic PKC (Billah and Anthes, 1990). Therefore, variations in the nuclear phospholipid content and relative composition may affect the functional activity of this key enzyme.

The main reports concerned with the demonstration of

phospholipids in the nucleus stem from fractionation studies, in which the possibility of a partial removal or displacement of phospholipids cannot be completely ruled out, even though the purified nuclear fractions have been convincingly shown to be free of membrane and cytoplasmic contaminations. We have recently demonstrated, by ultrastructural cytochemistry, the presence of phospholipids in defined nuclear domains in whole cells and in tissues (Maraldi et al., 1992b). These nuclear domains, the interchromatin fibers and granules, and the nucleolus, are sites in which transcription products are processed and they belong to the nuclear matrix compartment, with which PKC and some enzymes of the inositol lipid cycle are also associated (Capitani et al., 1987; Payrastre et al., 1991).

It seems thus worthwhile to determine whether, in association with DNA replication, changes in the amount and localization of nuclear phospholipids occur. By *in situ* ultrastructural cytochemistry, we show here that the nuclear phospholipids decrease in rat liver nuclei in which, as revealed by bromodeoxyuridine (BrdU) incorporation, DNA replication in response to partial hepatectomy takes place.

MATERIALS AND METHODS

Partial hepatectomy and BrdU incorporation

Male Wistar rats (150-200 g) were operated on as previously reported (Mazzoni et al., 1992b). Rats were injected intraperitoneally with bromodeoxyuridine (BrdU; 25 mg/kg body weight) 1 h before being killed, which was done 22 h after hepatectomy, in correspondence with the maximum uptake of BrdU into DNA, as monitored by flow cytometry (Vitale et al., 1989).

Cytochemical labelings

Histological sections (10 μm thick) were incubated with a monoclonal antibody (MoAb) against BrdU (mouse IgG; Becton-Dikinson, Mountain View, CA) and with goat anti-mouse (GAM) IgG linked to 10 nm colloidal gold (cg) particles and then intensified with the Silver Enhancer Kit (Janssen Life Science, Beerse, Belgium).

For electron microscopy immunocytochemistry, liver samples were immediately fixed in 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 1 h at 4°C, dehydrated in ethanol and embedded in Epon. Thin sections, mounted on nickel grids, were treated, on one side, by floating them on drops of the following media: 10% H_2O_2 for 10 min etching; 5% normal goat serum for 30 min; anti-BrdU MoAb (1:100) in 0.05 M Tris-HCl, pH 7.6, containing 0.1% bovine serum albumin (BSA) overnight at 4°C; GAM IgG 10 nm cg (1:10) in 0.02 M Tris-HCl, pH 8.2, containing 0.1% BSA for 1 h (Mazzotti et al., 1990). The grids were then incubated on the opposite side for the detection of phospholipids by phospholipase A_2 (PLA_2)-gold complex essentially as previously described (Maraldi et al., 1992b). Briefly, the grids were treated with 0.5% ovalbumin in PBS, pH 8.0, for 10 min and then incubated with PLA_2-17 nm cg for 30 min. The grids were then stained with uranyl acetate and lead citrate.

Controls for BrdU consisted of samples not incubated with the primary antibody or incubated with a non-immune mouse serum instead of the primary antibody (Mazzotti et al., 1990). The controls for PLA_2 consisted of samples to which PC (12 mg/ml) was added as substrate for the PLA_2 gold complex (1:1, v/v) prior to the incubation and of samples treated with non-conjugated gold solution (Maraldi et al., 1992b).

Quantitative analysis of the label distribution

In regenerating liver thin sections, both DNA-replicating cells and quiescent cells were present side by side; the label due to the presence of BrdU (cg particles of 10 nm) identified the cells in which DNA replication occurred, and its distribution pattern allowed us to dissect the S phase. The numbers of cells which were either BrdU positive or BrdU negative were determined on large fields of histological sections, while the different steps of S phase were identified by electron microscopy. The quantitative evaluation of the PLA_2-gold complex labeling (cg particles of 17 nm), which was present in both BrdU positive and BrdU negative nuclei, was performed according to the following criteria: the comparison between resting and replicating nuclei was done on the same sample, in which the experimental conditions were identical; the cg grain count was done manually, since densitometric detection by image analysis also included metal precipitates, which often occur on the thin sections; computer-assisted image analysis was employed to determine the relative areas of the three nuclear domains, i.e. heterochromatin, interchromatin and nucleolus; BrdU positive cells were separately considered according to the labeling pattern for BrdU, identified as early, middle and late S phase (Mazzotti et al., 1990; Rizzoli et al., 1992); the results were expressed as absolute density, i.e. number of particles counted per μm^2 of the nuclear domain considered.

RESULTS

Identification of resting and cycling cells and dissection of the S phase

At 22 h after partial hepatectomy, a peak of incorporation of BrdU into DNA was revealed with an anti-BrdU antibody, by flow cytometry, as previously described (Vitale et al., 1989) (data not reported). At the same time, histological sections, in which BrdU incorporation was revealed by gold-conjugated secondary antibody and silver intensification, showed that about 30% of the cells exhibited characteristic nuclear labeling which appeared diffused, spotted on the inner and perinuclear chromatin, or concentrated as a ring at the nuclear periphery (Fig. 1). At the ultrastructural level, these three main patterns, corresponding to early, middle and late S, as previously demonstrated (Mazzotti et al., 1990), could be identified as well (Fig. 2). Neighbouring resting cells, which did not incorporate BrdU into DNA, were essentially unlabeled, except for the inner space of some mitochondria. The cells with no nuclear labeling, corresponding to resting cells, constituted an internal reference with which to compare the amount of nuclear phospholipids detectable in neighbouring identically treated cycling cells.

Cell cycle-related nuclear phospholipid changes

The concomitant use of the labeling for DNA and for phospholipids, employing gold particles of different size, allowed the detection of changes in the phospholipid content and localization in nuclei along with the cell cycle.

A comparison among resting hepatocytes and those incorporating BrdU into DNA at different moments of the S phase is reported in Figs 3A,B and 4A,B. In the nucleus of hepatocytes not incorporating BrdU (Fig. 3A) the only label present was that detecting nuclear phospholipids (PLA_2-17 nm gold). The labeling was more intense in the interchromatin than in other nuclear domains (Fig. 3A). In

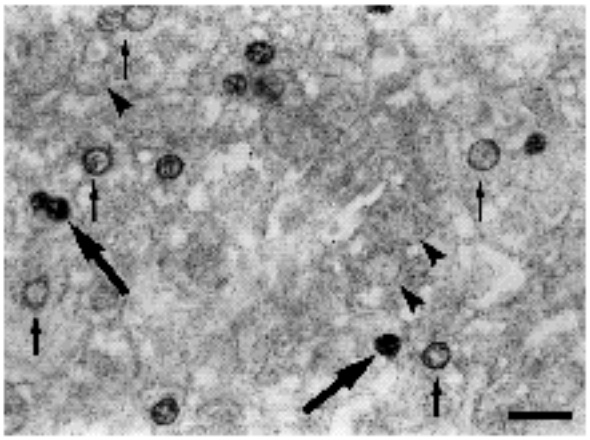

Fig. 1. Regenerating rat liver paraffin-embedded section; hematoxylin staining; anti-BrdU MoAb, GAM IgG 10 nm cg; silver enhancement. Some nuclei with no labeling ($BrdU^-$) are indicated by arrowheads; among the $BrdU^+$ nuclei, some display a diffused labeling (large arrows), other a ring-shaped labeling (small arrows). Intermediate types of labeling are also present. Bar, 15 μm.

the nucleus of BrdU-incorporating cells (Fig. 3B) 10 nm cg particles appeared mainly in the interchromatin, either scattered or clustered in distinct replicon domains, corresponding to a typical early S pattern. The PLA_2 labeling appeared reduced with respect to resting hepatocytes (Fig. 3B). Similar reduction in the PLA_2 labeling occurred in hepatocytes exibiting a middle S pattern (Fig. 4A) and a late S pattern of BrdU incorporation (Fig. 4B).

The quantitative evaluation of the label density indicated a significant reduction in the phospholipid label in nuclei of BrdU positive compared to BrdU negative cells (Table 1). This drop occurred in all nuclear domains, but was higher in interchromatin than in the nucleolus and heterochromatin, with a reduction of 50% and 30%, respectively, throughout all the steps of the S phase (Table 1).

Table 1. Quantitative distribution of labeling with phospholipase A_2-gold in resting ($BrdU^-$) and cycling ($BrdU^+$) cells

		$BrdU^+$		
Site	$BrdU^-$	Early S	Middle S	Late S
N	11.3 ± 1.9	9.2 ± 2.2	6.8 ± 1.0	7.2 ± 2.0
HC	2.9 ± 1.3	1.7 ± 2.3	1.8 ± 1.5	2.2 ± 1.3
IC	19.8 ± 2.9	10.8 ± 0.5	9.7 ± 1.0	9.8 ± 0.8

The values are expressed as density, i.e. number of particles per μm^2 ± s.d. (n = 30). In cycling cells ($BrdU^+$), the different steps of the S phase have been identified by characteristic patterns of the anti-BrdU labeling. N, nucleolus; HC, heterochromatin; IC, interchromatin.

DISCUSSION

The amount and composition of the nuclear phospholipids have long been reported to vary in relationship with key metabolic events and mainly with cell proliferation. Increased amounts of phospholipids associated with the chromatin have been found in hepatoma nuclei, compared to normal liver (Coetzee et al., 1975); variations in the relative composition of the chromatin-associated phospholipids occurred in chronic lymphocytic leukemia cells compared to normal B lymphocytes (Manzoli et al., 1977), during the cell cycle in HeLa cells (Alesenko and Burlakova, 1976), and in regenerating liver after partial hepatectomy (Alesenko and Pantaz, 1983). The neutral lipid/phospholipid ratio increased in Ehrlich ascites tumour with respect to normal cells (Awad and Spector, 1976; Balint and Holczinger, 1978); the treatment with insulin-like growth factor I (IGF-I) of mouse Swiss 3T3 cells caused a transient decrease in nuclear inositol lipid phosphorylation (Cocco et al., 1988, 1989); changes in nuclear inositol lipid phosphorylation occurred in Friend erythroleukemia cells (FELC) induced to erythroid differentiation (Cocco et al., 1987; Capitani et al., 1990, 1991). Consistent with these data are the findings that a discrete subcellular localization of the phosphoinositidases β, γ and δ occurs in different cell types, and that the β isoform predominates in the nucleus (Martelli et al., 1992; Mazzoni et al., 1992a).

The results reported here have been obtained by avoiding the cell manipulation required for fractionation studies, and are virtually unaffected by possible drawbaks such as non-homogeneous or poorly synchronized cells, or artifactual

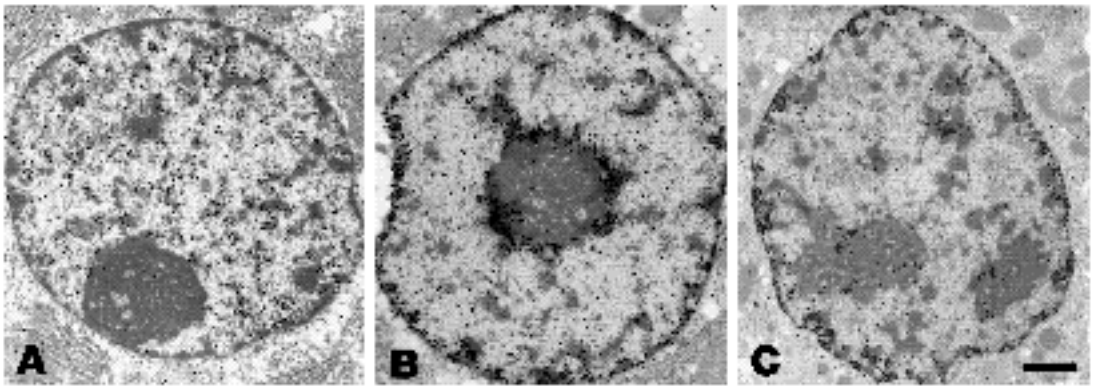

Fig. 2. (A-C) Regenerating rat liver thin section; anti-BrdU MoAb, GAM IgG 10 nm cg, silver enhancement. Three typical labeling patterns are shown: diffused, corresponding to early S (A); localized at the border of heterochromatin and at the periphery of the nucleolus, corresponding to middle S (B); confined to the peripheral heterochromatin, corresponding to late S (C). Bar, 1 μm.

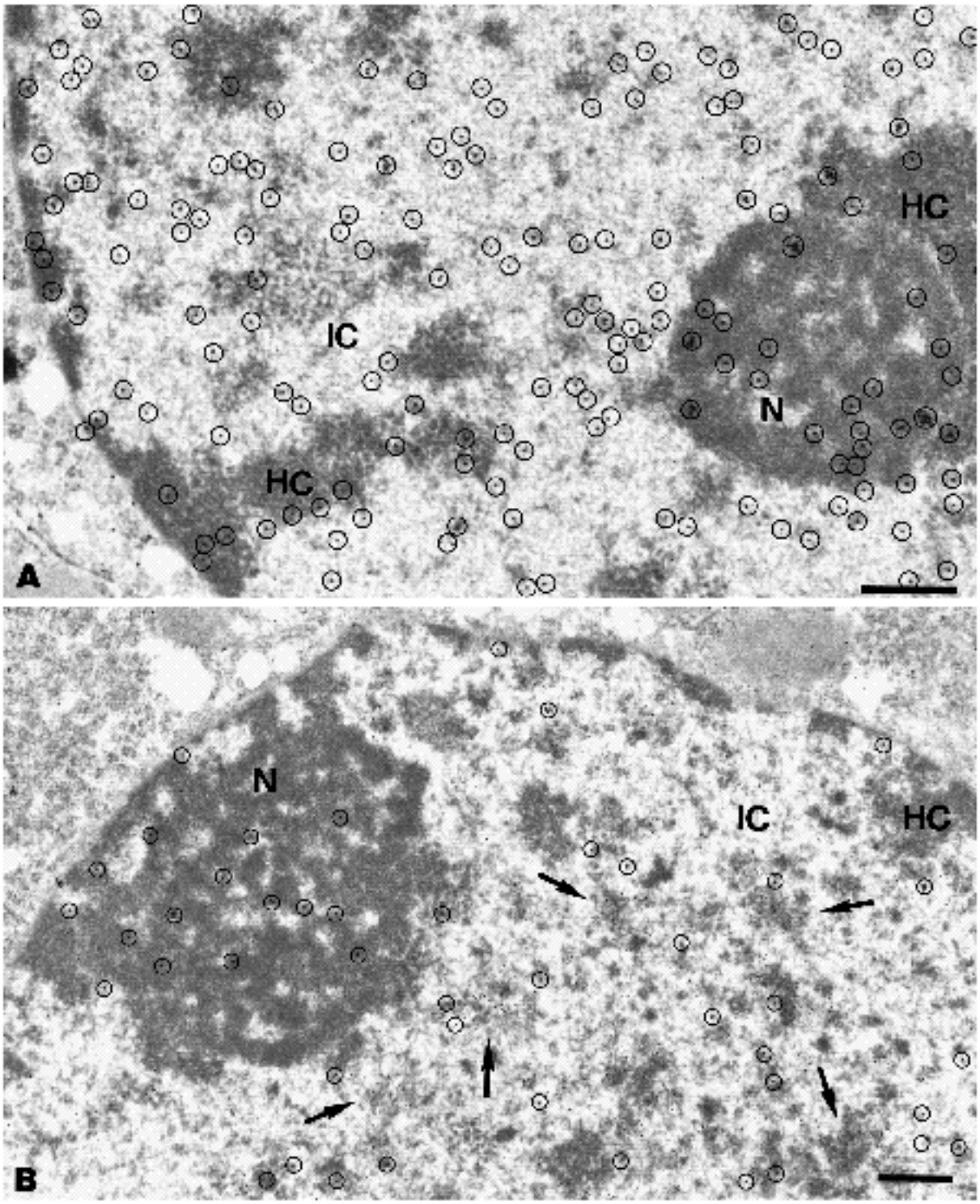

Fig. 3. (A,B) Regenerating rat liver thin section; double labeling: anti-BrdU MoAb, GAM IgG 10 nm cg, PLA_2-gold complex (cg 17 nm). (A) In a resting hepatocyte, the nucleus does not present labeling for BrdU, while the PLA_2-gold complex (encircled) labels the nucleoplasm, mainly in the interchromatin (IC) and nucleolar (N) domains and at the periphery of the heterochromatin (HC). (B) In a hepatocyte nucleus carrying on DNA synthesis, the labeling for BrdU is mainly localized, either diffused or spotted, in interchromatin areas (arrows) with a pattern typical of early S. The amount of PLA_2-gold complex labeling (encircled) appears reduced with respect to BrdU negative cells in all the nuclear domains and, to a larger extent, in the interchromatin (IC). Bar, 0.5 μm.

degradation. Our data indicate variations in the nuclear phospholipid content and localization at the level of single cells, with respect to neighbouring cells in the same tissue sample and under the same experimental conditions. A reduction in nuclear phospholipids occurs throughout the entire S phase, suggesting that this lipid modulation is not restricted to the

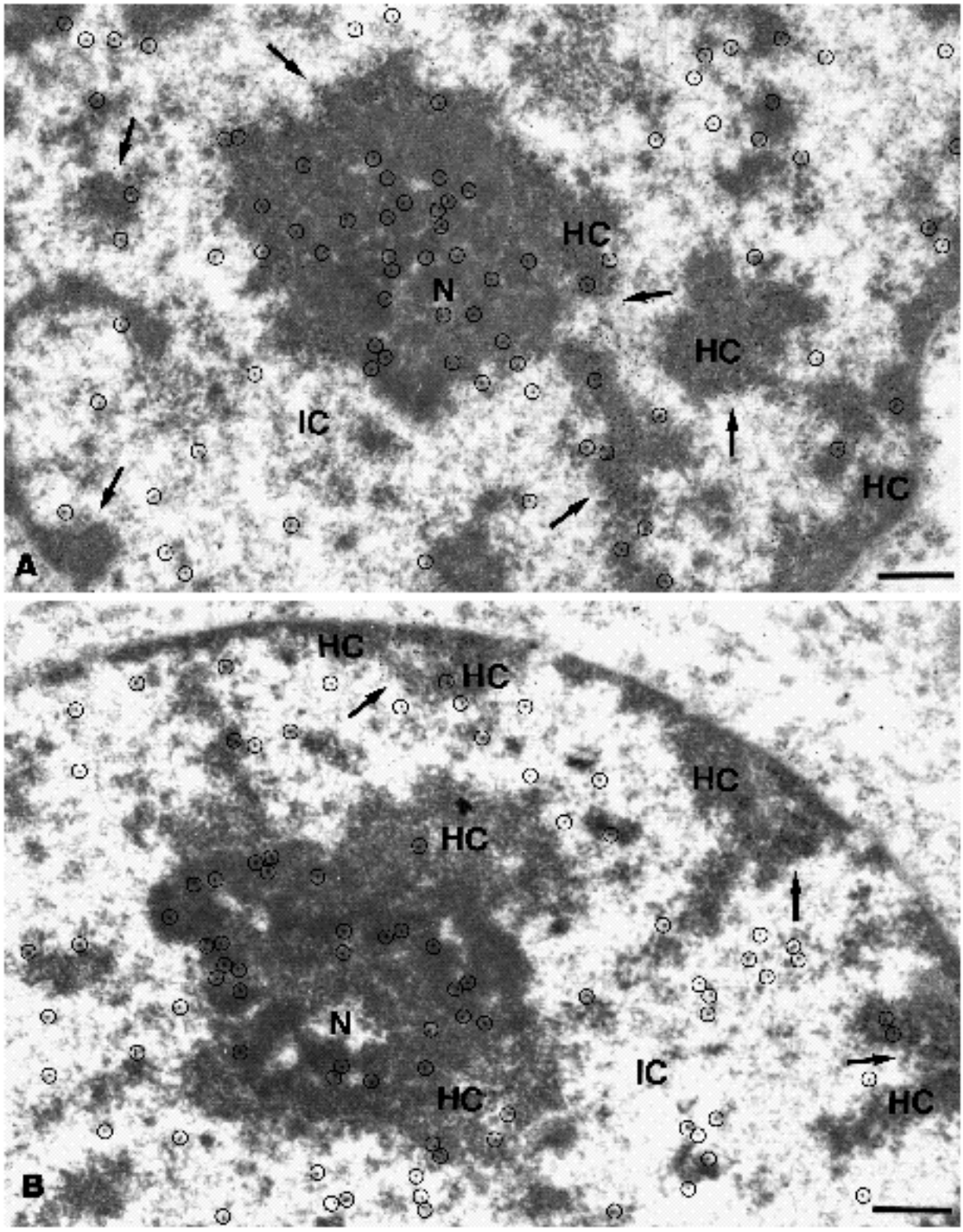

Fig. 4. (A,B) Regenerating rat liver thin section; double labeling: anti-BrdU MoAb, GAM IgG 10 nm cg, PLA_2-gold complex (cg 17 nm). (A) The hepatocyte nucleus shows a typical middle S pattern with the label for BrdU present along the inner masses of heterochromatin (HC, arrows). A reduced amount of PLA_2-gold complex labeling (encircled) with respect to BrdU negative nuclei (Fig. 3A) is present in all the nuclear domains. (B) A similar reduction in the PLA_2-gold complex (encircled) occurs in a hepatocyte nucleus with a typical late S pattern on the peripheral clumps of heterochromatin (HC, arrows). Bar, 0.5 μm.

onset of DNA synthesis as a trigger event. The constancy of the values observed in the three stages of the S phase indicates that the phenomenon is required for each replication wave, which occurs in different nuclear compartments involving different levels of chromatin compaction.

The PLA_2-gold method has been compellingly validated for the study of quantitative variations in the total phospholipid content in electron microscope sections. In fact, quantitative variations in labelling have been reported in different membrane regions, known to present a variable phospholipid content (Coulombe and Bendayan, 1989a) or in experimental conditions which affect the phospholipid accumulation (Coulombe and Bendayan, 1989b). In addition, we have previously shown that the PLA_2-gold system used identifies all phospholipid classes used as pure liposomes, and its efficiency is greater for the phospholipids of the nucleus, conceivably forming lipoprotein complexes, than for the membrane phospholipids arranged in bilayer leaflets (Maraldi et al., 1992b). Therefore, the system appears to be suitable for detecting variations in the total phospholipid amount in the nucleus, though a different affinity of the phospholipids for nuclear proteins or nucleoproteins during the S phase cannot be completely ruled out. In regenerating rat liver, an increased amount of the hydrolysis products of phosphoinositides has been found (Kurichi et al., 1992). Preliminary results on rat liver nuclei after partial hepatectomy confirm that an increased cleavage of phosphatidylinositol 4,5-bisphosphate (PIP_2) occurs and that also PC is degraded to phosphorylcholine (Capitani et al., unpublished data). These data suggest that regenerating liver nuclei express specific variations in activity of lipid phosphomonoesterases and phosphodiesterases. The increased activity of these enzymes could produce a rise in the levels of diacylglicerol and inositol trisphosphate (IP_3), which, in turn, could modulate calcium-dependent nuclear PKC activity. The reported activation of nuclear PKC in regenerating liver (Okamoto et al., 1988; Sasaki et al., 1990) has been suggested to affect the phosphorylation levels of some nuclear-specific substrates (Martelli et al., 1991b; Mazzoni et al., 1992b).

The phosphorylation of specific nuclear substrates, such as chromatin proteins by PKC, or the direct effect of IP_3 on intranuclear calcium level, may therefore be considered as a key regulatory element in the initial steps of cell proliferation (Irvine and Divecha, 1992).

In conclusion, the data here reported further support the contention that phospholipids and enzymes related to their breakdown are involved in the generation of signal molecules in the nucleus, which, eventually, induce DNA replication. However, comprehensive understanding of the cascade of events which follows the intranuclear accumulation of the products of the inositide and PC hydrolysis, such as diacylglicerol and inositol phosphates, requires further studies.

We thank Mr. Aurelio Valmori for skilful photographic assistance. This work was supported by grants from Italian C.N.R. PFIG/PFACRO and by Prog. Ric. San. Fin. 1988-1990.

REFERENCES

Alesenko, A. V. and Burlakova, E. B. (1976). Role of phospholipids in DNA synthesis in mammalian cells. *Doklady Akademy Nauk USSR* **229**, 299-310.

Alesenko, A. V. and Pantaz, E. A. (1983). Differences in the composition of nuclear and chromatin phospholipids in proliferating rat liver cells after partial hepatectomy. *Biochemia* **48**, 263-268.

Awad, A. B. and Spector, A. A. (1976). Modification of the Ehrlich ascite tumor cell nuclear lipids. *Biochim. Biophys. Acta* **450**, 239-251.

Azhar, S., Butte, J. and Reaven, E. (1987). Calcium-activated, phospholipid-dependent protein kinase from rat liver: subcellular distribution, purification, and characterization of multiple forms. *Biochemistry* **26**, 7047-7057.

Balint, Z. and Holczinger, E. (1978). Neutral lipids in nuclei and chromatin fraction of young and old Ehrlich ascites tumor cells. *Neoplasma* **1**, 25-32.

Billah, M. M. and Anthes, J. C. (1990). The regulation and cellular functions of phosphatidylcoline hydrolysis. *Biochem. J.* **269**, 281-291.

Capitani, S., Billi, A. M., Bertagnolo, V., Previati, M., Mazzoni, M., Neri, L. M. and Manzoli, F. A. (1991). Inositol lipids in Friend erythroleukemia cells: evidence for changes in nuclear metabolism after differentiation. *Cell Biochem. Funct.* **9**, 135-145.

Capitani, S., Girard, P. R., Mazzei, G., Kuo, J. F., Berezney, R. and Manzoli, F. A. (1987). Immunochemical characterization of protein kinase C in rat liver nuclei and subnuclear fractions. *Biochem. Biophys. Res. Commun.* **142**, 367-375.

Capitani, S., Mazzoni, M., Bertagnolo, V., Previati, M., Daddona, A., Ricci, D. and Manzoli, F. A. (1990). Nuclear inositol lipids in Friend erythroleukemia cells. Changes related to differentiation induced by hexamethylenebisacetamide. *Cell Biol. Int. Rep.* **14**, 783-795.

Cocco, L., Gilmour, R. S., Ognibene, A., Letcher, A. J., Manzoli, F. A. and Irvine, R. F. (1987). Synthesis of polyphosphoinositides in nuclei of Friend cells. Evidence for polyphosphoinositides metabolism inside the nucleus which changes with cell differentiation. *Biochem. J.* **248**, 765-770.

Cocco, L., Martelli, A. M., Gilmour, R. S., Ognibene, A., Manzoli, F. A. and Irvine, R. F. (1988). Rapid changes in phospholipid metabolism within nuclei of Swiss 3T3 cell induced by treatment of the cell with insulin-like growth factor I. *Biochem. Biopys. Res. Commun.* **154**, 1266-1272.

Cocco, L., Martelli, A. M., Gilmour, R. S., Ognibene, A., Manzoli, F. A. and Irvine, R. F. (1989). Changes in nuclear inositol phospholipids induced in intact cells by insulin-like growth factor I. *Biochem. Biophys. Res. Commun.* **159**, 720-725.

Coetzee, M. L., Sprangler, M., Morris, H. P. and Ove, P. (1975). DNA synthesis in membrane-denuded nuclei and nuclear fractions from host liver and Morris Hepathomas. *Cancer Res.* **35**, 2752-2761.

Coulombe, P. A. and Bendayan, M. (1989a). Heterogeneous distribution of phospholipids in membranes along the secretory pathway in pancreatic β-cells. *Biol. Cell* **65**, 83-86.

Coulombe, P. A. and Bendayan, M. (1989b). Cytochemical demonstration of increased phospholipid content in cell membranes in chlorophentermine-induced phospholipidosis. *J. Histochem. Cytochem.* **37**, 139-147.

Cruise, J. L., Houck, K. A. and Michalopoulus, G. (1985). Inducing of DNA synthesis in coltured rat hepatocytes through stimulation of alpha 1 adrenoreceptor by norepinephrine. *Science* **227**, 749-751.

Cruise, J. L., Knechtle, S. J., Bollinger, R. R., Kuhn, C. and Michalopoulus, G. (1987). Alpha 1-adrenergic effects and liver regeneration. *Hepatology* **7**, 1189-1194.

Divecha, N., Banfic, H. and Irvine, R. F. (1991). The polyphosphoinositide cycle exists in the nuclei of Swiss 3T3 cells under the control of a receptor (for IGF-I) in the plasma membrane and stimulation of the cycle increases nuclear diacylglicerol and apparently induces translocations of protein kinase C to the nucleus. *EMBO J.* **10**, 3207-3214.

Irvine, R. F. and Divecha, N. (1992). Phospholipids in the nucleus. Metabolism and possible functions. *Semin. Cell Biol.* **3**, 225-235.

Kurichi, H., Tamiya-Koizumi, K., Asano, M., Yoshida, S., Kojima, K. and Nimura, Y. (1992). Existence of phosphoinositide-specific phospholipase C in rat liver nuclei and its change during liver regeneration. *J. Biochem.* **111**, 283-286.

Laks, M. S., Harrison, J. J., Schwoch, G. and Jungmann, R. A. (1981). Modulation of nuclear protein kinase activity and phosphorylation of histone H1 subspecies during the prereplicative phase of rat liver regeneration. *J. Biol Chem.* **256**, 8775-8785.

Manzoli, F. A., Maraldi, N. M., Cocco, L., Capitani, S. and Facchini, A. (1977). Chromatin phospholipids in normal and chronic lymphatic leukemia lymphocytes. *Cancer Res.* **37**, 843-849.

Maraldi, N. M., Mazzotti, G., Capitani, S., Rizzoli, R., Zini, N., Squarzoni, S. and Manzoli, F. A. (1992a). Morphological evidence of function related localization of phospholipids in the cell nucleus. *Advan. Enzyme Regul.* **32**, 73-90.

Maraldi, N. M., Zini, N., Squarzoni, S., Del Coco, R., Sabatelli, P. and Manzoli, F. A. (1992b). Intranuclear localization of phospholipids by ultrastructural cytochemistry. *J. Histochem. Cytochem.* **40**, 1383-1392.

Martelli, A. M., Carini, C., Marmiroli, S., Mazzoni, M., Barker, P. J., Gilmour, R. S. and Capitani, S. (1991a). Nuclear protein kinases in rat liver: evidence for increased histone H1 phosphorylation activity during liver regeneration. *Exp. Cell Res.* **195**, 255-262.

Martelli, A. M., Gilmour, R. S., Bertagnolo, V., Neri, L. M., Manzoli, L. and Cocco, L. (1992). Nuclear localization and signalling activity of phosphoinositidase C β in Swiss 3T3 cells. *Nature* **358**, 242-245.

Martelli, A. M., Neri, L. M., Gilmour, R. S., Barker, P. J., Huskisson, N. S., Manzoli, F. A. and Cocco, L. (1991b). Temporal changes in intracellular distribution of protein kinase C in Swiss 3T3 cells during mitogenic stimulation with insulin like growth factor I and bombesin: translocation to the nucleus follows rapid changes in nuclear polyphosphoinositides. *Biochem. Biophys. Res. Commun.* **177**, 480-487.

Mazzoni, M., Bertagnolo, V., Neri, L. M., Carini, C., Marchisio, M., Milani, D., Manzoli, F. A. and Capitani, S. (1992a). Discrete subcellular localization of phosphoinositidase C β, γ and δ in PC12 rat pheochromocytoma cells. *Biochem. Biophys. Res. Commun.* **187**, 114-120.

Mazzoni, M., Carini, C., Matteucci, A., Martelli, A. M., Bertagnolo, V., Previati, M., Rana, R., Cataldi, A. and Capitani, S. (1992b). Increased phosphorylation of nuclear substrates for rat brain protein kinase C in regenerating rat liver nuclei. *Cell. Signalling* **4**, 313-319.

Mazzotti, G., Rizzoli, R., Galanzi, A., Papa, S., Vitale, M., Falconi, M., Neri, L. M., Zini, N. and Maraldi, N. M. (1990). High-resolution detection of newly synthesized DNA by anti-bromodeoxyuridine antibodies identifies specific chromatin domains. *J. Histochem. Cytochem.* **38**, 13-22.

Michell, R. H. (1992). Nuclear PIPs. *Curr. Biol.* **2**, 200-202.

Okamoto, Y., Nishimura, K., Nakayama, M., Nakagawa, M. and Nakano, H. (1988). Protein kinase C in the regenerating rat liver. *Biochem. Biophys. Res. Commun.* **151**, 1144-1149.

Olsen, P. S., Boesby, S., Kirkegard, P., Therkelson, K., Almdal, T., Poulsen, S. S. and Nexo, E. (1988). Influence of epidermal growth factor on liver regeneration after partial hepatectomy in rats. *Hepatology* **8**, 992-996.

Payrastre, B., van Bergen en Henegouwen, P. M. P., Breton, M., den Hartigh, J. C., Plantavid, M., Verkleij, A. J. and Boonstra, J. (1991). Phosphoinositide kinase, diacylglycerol kinase, and phospholipase C activities associated to the cytoskeleton: effect of epidermal growth factor. *J. Cell Biol.* **115**, 121-128.

Rizzoli, R., Baratta, B., Maraldi, N. M., Falconi, M., Galanzi, A., Papa, S., Vitale, M., Rizzi, E., Manzoli, L. and Mazzotti, G. (1992). DNA synthesis progression in 3T3 synchronized fibroblasts: a high resolution approach. *Histochemistry* **97**, 181-187.

Rogue, P., Labourdette, G., Masmoudi, A., Yoshida, Y., Huang, F. L., Huang, K. P., Zwiller, J., Vincendon, G. and Malviya, A. N. (1990). Rat liver nuclei protein kinase C is the isozyme type II. *J. Biol. Chem.* **265**, 4161-4165.

Sasaki, Y., Hayashi, N. and Kamada, T. (1990). Heterogeneous activation of protein kinase C during rat liver regeneration. *Adv. Second Messengers Phosphoprot. Res.* **24**, 345-351.

Vitale, M., Neri, L. M., Manzoli, L., Galanzi, A., Rana, R., Antonucci, A. and Papa, S. (1989). Improved bromodeoxyuridine DNA analysis by anti-BudR monoclonal antibody versus right angle light scatter. *Histochemistry* **93**, 9-11.

(Received 21 September 1992 - Accepted 30 November 1992)