Presence and Activation of Nuclear Phosphoinositide 3-Kinase C2β during Compensatory Liver Growth*

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Highly purified liver nuclei incorporated radiolabeled phosphate into phosphatidylinositol 4-phosphate (PtdIns(4)P), PtdIns(4,5)P2, and PtdIns(3,4,5)P3. When nuclei were depleted of their membrane, no radiolabeling of PtdIns(3,4,5)P3 could be detected showing that within the intranuclear region there are no class I phosphoinositide 3-kinases (PI3Ks). In membrane-depleted nuclei harvested 20 h after partial hepatectomy, the incorporation of radiolabel into PtdIns(3)P was observed together with an increase in immunoprecipitable PI3K-Cβ activity, which is sensitive to wortmannin (10 nM) and shows strong preference for PtdIns over PtdIns(4)P as a substrate. On Western blots PI3K-Cβ revealed a single immunoreactive band of 180 kDa, whereas 20 h after partial hepatectomy gel shift of 18 kDa was noticed, suggesting that observed activation of enzyme is achieved by proteolysis. When intact membrane-depleted nuclei were subjected to short term (20 min) exposure to μ-calpain, similar gel shift together with an increase in PI3K-Cβ activity was observed, when compared with the nuclei harvested 20 h after partial hepatectomy. Moreover, the above-mentioned gel shift and increase in PI3K-Cβ activity could be prevented by the calpain inhibitor calpeptin. The data presented in this report show that, in the membrane-depleted nuclei during the compensatory liver growth, there is an increase in PtdIns(3)P formation as a result of PI3K-Cβ activation, which may be a calpain-mediated event.

The phosphorylation and hydrolysis of phosphoinositid lipid is a major pathway for the generation of a diverse set of second messenger signaling molecules. A phosphatidylinositol-based phosphorylation/hydrolysis cycle has been identified in the plasma membrane, which plays a critical role in the activation of several serine/threonine protein kinases, including protein kinase C (PKC)1 and protein kinase B (PKB) or Akt. Although the presence and activation of phospholipase C (PLC) in the cell nucleus has been extensively documented (1–3), the presence of phosphoinositide 3-kinase (PI3K) has been shown only recently (4–7). On the basis of their structure and in vitro activity, PI3K isoforms have been divided into three classes termed I, II, and III (8). Class I enzymes utilize PtdIns, PtdIns(4)P and PtdIns(4,5)P2, as a substrate in vitro to produce their 3-phospholipid products from which PtdIns(3,4)P2 and PtdIns(3,4,5)P3 are able to activate PKB in vivo (9–11). In the cell nucleus both subclasses of class I have been shown to exist, subclass IA, which binds p85-adapter that facilitates translocation to phosphotyrosine-containing signaling complexes, and subclass IB, which contains the G-protein-activated enzyme p110γ (4–7). Class II PI3K enzymes are distinguished from other PI3K isoforms by the presence of two tandem domains in their carboxy terminus. The first one is termed a phox homology domain, and the function of this domain is rather unclear (12), whereas the second is the C2 domain, which is a phospholipid-binding domain that can confer a Ca2+ sensitivity (13). All three members of the class II PI3K enzymes (PI3K-Cα, PI3K-Cβ, and PI3K-Cγ) are able to phosphorylate PtdIns and PtdIns(4)P in vitro assays, but the mechanism of their activation and the function of their 3-phosphoinositide products in vivo are poorly understood. However, PI3K-Cα plays a signaling role downstream of monocyte chemoattractant peptide receptor 1 (14) and insulin receptor (15) and is concentrated in the trans-Golgi network and present in clathrin-coated vesicles (16). In the platelets, PI3K-Cβ is activated in response to stimulation of integrin receptors by fibrinogen (17), whereas both PI3K-Cα and PI3K-Cβ are downstream signaling targets of activated epidermal growth factor and platelet-derived growth factor receptors (18). Although PI3K-Cα and PI3K-Cβ share a wide tissue distribution, PI3K-Cγ expression is restricted primarily to hepatocytes and is enhanced during the liver regeneration (19, 20). Class III PI3K contains only a single enzyme termed Vps34p, which in yeast regulates vacuolar trafficking through generation of PtdIns(3)P (21, 22). Using a well-described model of the liver regeneration in which we have previously demonstrated the activation of PLC with accompanied translocation of PKC to the nucleus (23), the present study was undertaken to investigate the metabolism of 3-phosphorylated phosphoinositides in the light of the recently demonstrated
compartmentalization of inositol lipids in the isolated liver nuclei (24).

**EXPERIMENTAL PROCEDURES**

Reagents were obtained from the following sources: EGTA, EDTA, HEPES, Tris, leupeptin, phenylmethylsulfonyl fluoride, phosphatidylserine, Triton X-100, Na⁺ deoxycholate, protein A-Sepharose, SDS, and aprotinin from Sigma Chemical Co., St. Louis MO; inositol lipids from Eschlon Research Laboratories, Salt Lake City, UT; wortmannin, μ-calpain, and calpeptin from Calbiochem, Nottingham, UK; [γ-³²P]ATP, [³⁵S]methionine, [³⁵S]cysteine, and enhanced chemiluminescence kit from Amersham Pharmacia Biotech, Bucks, UK. All other chemicals were of analytical grade.

Male Wistar rats (150–250 g of body wt) were used in all experiments. When partial heptectomy was performed two-thirds of the liver was surgically removed (23).

*Purification of Liver Nuclei—*Livers were collected on ice, washed twice in solution B (10 mM HEPES (pH 7.5), 5 mM MgCl₂, 25 mM KCl) and 14 g (wet wt) was homogenized in 28 ml of the same solution using a power-driven pestle. To this was added 4.9 ml of solution C (10 mM HEPES (pH 7.5), 2 mM MgCl₂, 2.5 mM sucrose), to give a final concentration of sucrose of 0.25 M. This was then mixed by inversion with solution D (10 mM HEPES (pH 7.5), 2 mM MgCl₂, 2.3 M sucrose; 90 ml), to give a final sucrose concentration of 1.62 M. A 7.5-ml cushion of solution D was then layered carefully below 23 ml of the final (1.62 M sucrose) liver homogenate. The samples were then spun at 106,000 g for 6 min (4 °C), and the supernatant was removed. The pellet was resuspended in 0.5 ml of solution A (24).

*Preparation of Membrane-depleted Nuclei—*A quantitative removal of the nuclear envelope was performed with the non-ionic detergent Triton X-100. A 0.5-ml aliquot of nuclei in solution A was mixed with 20 ml of ice-cold final resuspension buffer (5 mM Tris (pH 7.4), 5 mM MgCl₂, 1.5 mM KCl, 1 mM EDTA, and 0.25 μm sucrose). The fractions were then precipitated with a final concentration of 0.04% (w/v). The nuclei were pelleted at 165 × g for 6 min at 4 °C, and finally being resuspended to 4 ml in this solution (24). The purity of nuclei was estimated by the determination of marker enzymes (lecine aryldiastase, Na⁺-K⁺-ATPase, succinate:cytochrome c oxidoreductase, KCN-resistant NADH oxidoreductase, and 5-nucleotidase) and by electron microscopy (23, 24).

*Preparation of Cell Lysate, Cytosolic Fraction, and Postnuclear Membranes—*Cell lysate was prepared by homogenization of liver tissue in solution B as stated above. Cytosolic fraction was prepared by homogenization in solution B, and afterward samples were spun at 160,000 × g for 90 min at 4 °C in a Beckman SW 27 rotor, and clear supernatant was considered to be cytosolic fraction. Preparation of postnuclear membranes was achieved by centrifugation of supernatant, which remained above cushion after the nuclear fraction has been obtained. The supernatant was diluted in solution B to give a final concentration of 162 mM sucrose and spun at 106,000 × g for 90 min at 4 °C in a Beckman SW 27 rotor. The resultant pellet was considered to contain postnuclear membranes.

**Labeling of Inositol Lipids with [γ-³²P]ATP—**For the *in vitro* labeling of inositol lipids with [γ-³²P]ATP nuclei (total protein 100 μg) were resuspended in 90 μl of buffer containing 10 mM HEPES (pH 7.5), 5 mM MgCl₂, 1.5 mM KCl, 1 mM EDTA, and 0.25 μm sucrose. The samples were preincubated for 2 min at 30 °C to hydrolyze any remaining endogenous ATP. Then 10 μl of phosphorylation mixture (40 μCi of [γ-³²P]ATP, 2 μl of 5 mM non-radiolabeled ATP, made up to 10 μl with the above-mentioned buffer) was added. Incubation was carried out for 5 min at 30 °C and terminated as described above. Lipids were extracted and deacylated, and the separation of all the glycerophosphoinositides was achieved using an HPLC high resolution 5 μM Partisphere SAX column (Whatman) with a discontinuous gradient up to 1 M (NH₄)₂HPO₄ (pH 3.8) exactly as described in a previous study (25).

*Immunoprecipitation of P13K-C2β—*P13K-C2β isofrom-discriminant polyclonal antiserum against the first 331-amino acid portion of P13K-C2β (26), expressed in Escherichia coli as an amino-terminally fused glutathione S-transferase protein, were raised in rabbits as described previously (17). These antiseria were used for all immunoprecipitations and Western blots directed at P13K-C2β. Native or membrane-depleted nuclei were resuspended in 0.5 ml of buffer containing 50 mM Tris (pH 7.6), 150 mM NaCl, 1% Triton X-100 (w/v), 0.5% Na⁺ deoxycholate (w/v), 0.1% SDS (w/v), 2 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, and 1 μg/ml leupeptin and spun at 100,000 × g for 90 min at 4 °C. P13K-C2β was immunoprecipitated overnight from 450 μl of supernatants with antibody and protein A-Sepharose. Immunoprecipitates were washed once with the above-mentioned buffer, then three times with 5 mM HEPES/2 mM EDTA (pH 7.5) and then the phosphorylation assay was carried out as described above for exogenous lipid phosphorylation.

**Western Blot Analysis of P13K-C2β—**Proteins for electrophoresis

**Fig. 1.** Incorporation of ³²P into PtdIns(4)P, PtdIns(4,5)P₂, and PtdIns(3,4,5)P₃ in the intact and membrane-depleted liver nuclei and after addition of exogenous lipid to the membrane-depleted nuclei. Nuclei were prepared and radiolabeled as described under "Experimental Procedures." The results are means ± S.E. for three different experiments, each performed in duplicate. *, p < 0.05 (Student's t test) with respect to the intact nuclei. n.d., not detectable.
were prepared so that the concentration of each sample was 50 μg/25 μl of sample loading buffer (27), and electrophoresis was carried out using a Bio-Rad Minigel apparatus at an acrylamide concentration of 5% (w/v) or 12% (w/v) when expressed amino-terminal fragments of PI3K-C2β and PI3K-C2γ were run. After electrophoresis, the proteins were transferred to nitrocellulose using a Bio-Rad wet-blotting system. The blot was blocked with a buffer containing 4% (w/v) dried milk, 20 mM Tris, 140 mM NaCl, 0.05% (v/v) Tween 20. It was then probed for 2 h with primary antibody (1:1000), then washed with a blocking buffer and incubated with the secondary antibody conjugated to horseradish peroxidase. Visualization was carried out using the ECL kit (Amersham Pharmacia Biotech).

Expression of the Recombinant Amino-terminal Fragment of PI3K-C2γ—The amino-terminal fragment of PI3K-C2γ was amplified using PCR and a human liver cDNA library. Two nested pairs of oligonucleotides were used to produce a PCR template suitable for use in the STP3 in vitro transcription translation system (Novagen). The first PCR reaction produces a 1239-bp fragment from the 5'-region of PI3K-C2γ (5'-AACGGATCCAAATCCTAATGAAT at 118 and 3'-TCTGATGAGTGTTAAGAGACATTG at 1233). A T7 RNA polymerase promoter was incorporated in the sense primer of the second pair of oligonucleotides (GGATCCTAATACGACTCACTATAGGAAACAGACCACCATGAGCAGTATGAACACCAAG) along with a 3'-stop codon in the antisense primer (TCAAAACTGACTGTTGCTTCTTTC) used in the nested PCR. The protein expressed in the in vitro translation system spans the amino-terminal portion of PI3K-C2γ from amino acid 17 to amino acid 387, the region that overlaps to the antigenic portion of PI3K-C2β previously used as immunogen (28). 35S-Labeled methionine and cysteine were used to detect the in vitro translated PI3K-C2γ amino terminus.

Statistical Evaluation—The data are shown as means ± S.E. For statistical analyses, the Student’s t test for unpaired samples at the level of significance of 0.05 was used.

RESULTS
Assessment of Nuclear Purity—The purity of isolated nuclei was estimated by the determination of marker enzymes (see “Experimental Procedures”) and by electron microscopy (results not shown). Most marker enzymes were at levels so low that quantification was difficult, and electron microscopy showed no other obvious components present. The exception in this respect was for endoplasmic reticulum KCN-resistant NADH-oxidoreductase: the specific activity for this enzyme in nuclear preparations (original homogenate activity, 0.59 ± 0.04 μmol/mg of protein per ml) was 0.15 ± 0.02, which decreased to 0.08 ± 0.01 when the nuclei were depleted of membrane using 0.04% Triton X-100. The presence of KCN-resistant NADH oxidoreductase may be taken as evidence for residual endoplasmic reticulum contamination that is finally
removed by detergent, but it is equally likely that it is present in the nuclear membrane, which is also removed by detergent (23, 24).

Phosphorylation of Phosphoinositides in the Whole and Membrane-depleted Nuclei—As shown in Figs. 1 and 2 when the intact nuclei have been labeled for a short time period (5 min) the radioactivity was found in PtdIns(4)P, PtdIns(4,5)P₂, PtdIns(3,4,5)P₃ and PtdIns(3,4,5)P₃. As has already been noticed by Lu et al. (4) the formation of PtdIns(3,4)P₂ could only be found when incubation was carried out for 20 min or longer, therefore, in the present investigation the incubation time was too short to observe any PtdIns(3,4)P₂ formation. As shown in Fig. 1 the level of incorporation fell dramatically when the nuclei were depleted of their membrane, whereas no incorporation could be detected in

**Fig. 3.** Incorporation of ³²P into PtdIns(3)P, PtdIns(4)P, PtdIns(4,5)P₂, and PtdIns(3,4,5)P₃ in the intact nuclei (A), membrane-depleted nuclei (B), and membrane-depleted nuclei after addition of exogenous lipids (C). The nuclei were prepared and radiolabeled, and the separation of glycerophosphoinositides was achieved as described under “Experimental Procedures.” The control nuclei (open bars), nuclei harvested 20 h after partial hepatectomy (gray bars), the effect of wortmannin (10 nm) on nuclei harvested 20 h after partial hepatectomy (black bars) are shown. When exogenous lipid was added for measurement of incorporation of ³²P into PtdIns(4)P and PtdIns(3)P, PtdIns was used as a substrate; for PtdIns(4,5)P₂, PtdIns(4)P was used as substrate, while for PtdIns(3,4,5)P₃, PtdIns(4,5)P₂ was used as a substrate. All other details are as described under “Experimental Procedures.” The results are means ± S.E. for three different experiments, each performed in duplicate. * p < 0.05 (Student’s t test) with respect to membrane-depleted nuclei harvested 20 h after partial hepatectomy. n.d., not detectable.
PtdIns(3,4,5)P$_3$. This suggests that within the intranuclear region there are PtdIns and PtdIns(4)P available for phosphorylation, whereas there is no PtdIns(4,5)P$_2$ or that the 3-kinase is not present in the nuclei depleted of nuclear membrane. To address this question, exogenous lipid substrates were added, in the form of vesicles, to the membrane-depleted nuclei. Addition of exogenous lipid substrates resulted in the reconstitution of incorporation of phosphate into PtdIns(4)P and PtdIns(4,5)P$_2$ but not into PtdIns(3,4,5)P$_3$, suggesting that 3-kinase that utilizes PtdIns(4,5)P$_2$ as a substrate is not present in the membrane-depleted nuclei.

Because it is known that there is an increase in nuclear DAG concentration and PLC activity during the liver regeneration (23, 29), which peaks around 20 h following partial hepatectomy, the nuclei harvested at this time point were used for labeling isoinositol lipids (Fig. 2). Although the incorporation of radiolabeled phosphate into PtdIns(4)P, PtdIns(4,5)P$_2$, and PtdIns(3,4,5)P$_3$ did not change, radioactivity in PtdIns(3)P could be found. This was further documented in the membrane-depleted nuclei and membrane-depleted nuclei after addition of exogenous lipids (Fig. 3). Furthermore, this incorporation of phosphate into PtdIns(3)P is inhibitable by 10 nM wortmannin, suggesting the existence of 3-kinase in the membrane-depleted nuclei, which is unable to use PtdIns(4,5)P$_2$ as a substrate, because under the above-mentioned conditions no radioactivity could be found in PtdIns(3,4,5)P$_3$.

**Immunoprecipitation and Biochemical Characterization of Nuclear P13K-C2β**—Polyclonal antisera against the first 331-amino acid portion of P13K-C2β used in the immunoprecipitation studies do not detect class I PI3Ks, Vps34p, PI3K-C2b on Western blots or immunoprecipitates (17) and were used to further characterize the observed 3-kinase activity in the membrane-depleted nuclei. Despite the fact that there is absolutely no homology between amino-terminal portions of P13K-C2γ and P13K-C2β (19, 26), it is important to note that P13K-C2γ is expressed in hepatocytes and that its expression is enhanced during liver regeneration (19, 20); therefore, it is crucial that antisera used to immunoprecipitate P13K-C2β do not detect P13K-C2γ. As shown in Fig. 4 antisera used for immunoprecipitation and Western blotting of P13K-C2β were unable to detect the amino-terminal portion of P13K-C2γ, which overlaps the antigenic portion of P13K-C2β used as an immunogen (28).

Following partial hepatectomy, an increase in immunoprecipitable P13K-C2β activity could be observed in the membrane-depleted nuclei but not in total cell lysate, cytosolic fraction, or postnuclear membranes (Fig. 5). The time course of changes in immunoprecipitable P13K-C2β activity showed its maximum at 20 h after partial hepatectomy and then slowly declining until 32 h (Fig. 6), similar to the increase in nuclear DAG concentration and PLC activity (23, 29). It is important to note that no P13K-C2β activity could be found in the supernatant when the membrane-depleted nuclei were prepared (results not shown), suggesting that the enzyme is not present in the nuclear membrane.

Because it is known that P13K-C2β is able to phosphorylate PtdIns but not PtdIns(4)P in the presence of Ca$^{2+}$, whereas some phosphorylation of PtdIns(4)P could be observed in the presence of Mg$^{2+}$ (18, 28), in vitro substrate specificity for immunoprecipitable P13K-C2β was tested using Mg$^{2+}$ for phosphate transfer. As shown in Fig. 7 the basal level of PtdIns phosphorylation is about 4-fold higher than the phosphorylation of PtdIns(4)P, and this proportion increases to about 10-fold in the nuclei harvested 20 h after partial hepatectomy, showing that there is a strong preference for PtdIns over PtdIns(4)P as a substrate in both basal and stimulated conditions. Because it is known that 10 nM wortmannin completely inhibit stimulated P13K-C2β activity (28) whereas the IC$_{50}$ for P13K-C2γ inhibition is 32 nm, with maximal inhibition obtained with 10 μM wortmannin (19), the present observation that stimulated 3-kinase activity is inhibitable by 10 nM wortmannin further demonstrates that P13K-C2β is present in the nuclei and activated during compensatory liver growth.

**Proteolytic Activation of P13K-C2β**—Western blotting of cell
lysates and membrane-depleted nuclei, fractionated by SDS-PAGE on a 5% gels and probed with antisera raised against PI3K-C2β, revealed a single immunoreactive band of 180 kDa (Fig. 8). On the other hand, 20 h after partial hepatectomy a gel shift of 18 kDa was observed only in the membrane-depleted nuclei, suggesting that the observed activation of enzyme (Fig. 5) is achieved by proteolysis. It is important to point out that no proteolytic fragment (Fig. 8) and no increase in enzyme activity (Fig. 5) were detected in cell lysates that also contain nuclei. Therefore, this suggests that proteolysis and activation occur only in the absence of the cell membrane and/or cytosolic fractions, which in turn suggests the presence of an “inhibitor” of proteolysis in the latter cellular fractions. The fact that 50 µg of protein of total cell lysate and 50 µg of nuclear protein (Fig. 8) contain virtually the same amount of PI3K-C2β indicates that the vast majority of cellular PI3K-C2β is nuclear. Thus, proteolysis and/or activation of PI3K-C2β appear to require not only the effects of hepatectomy but also the process of isolating membrane-depleted nuclei.

Previously, we showed that a similar pattern of activation of PI3K-C2β in platelets could be prevented by calpain inhibitors calpeptin or calpain I inhibitor (17). Therefore, the intact membrane-depleted nuclei were subjected to short term exposure (20 min) to µ-calpain and similar gel shift together with the increase in PI3K-C2β activity was observed (Fig. 9), when compared with nuclei harvested 20 h after partial hepatectomy (Figs. 5 and 8). Moreover, the above-mentioned gel shift and the increase in PI3K-C2β activity could not be observed when the nuclei were incubated with Ca2+ alone and could be prevented in the presence of calpain inhibitor calpeptin, further suggesting that calpain-mediated proteolysis of the enzyme may be responsible for its activation.

**DISCUSSION**

The presence of both subunits of class I PI3K has been demonstrated in the cell nuclei. Although the p110γ subunit is translocated to the nuclear membrane from cytosol upon stim-
membrane-depleted nuclei exposed to calpain; lane 3 (Student’s a depleted nuclei incubated with calpain in the presence of calpeptin. The position of the molecular mass marker for tions with equimolar concentration of substrates, PtdIns(4)P 6 of the membrane-depleted nuclei to calpain. Results are means S.E. for three different experiments, each performed in duplicate. *, p < 0.05 (Student’s t test) with respect to the control. For Western blot analysis, the same experiments were performed, and protein (50 μg) was subjected to SDS-PAGE, transferred to nitrocellulose, and probed with anti-PI3K-C2 antibody: lane 1, control membrane-depleted nuclei; lane 2, membrane-depleted nuclei exposed to calpain; lane 3, membrane-depleted nuclei incubated only in the presence of Ca 2+; lane 4, membrane-depleted nuclei incubated with calpain in the presence of calpeptin. The position of the molecular mass marker for α 2-macroglobulin (180 kDa) is indicated on the left side by the arrow.

ulation of HepG2 cells with serum (7), translocation and pre-cise intranuclear localization of p85 subunit differs with cell types. In HL-60 cells it is localized in the nuclear matrix during granulocytic or monocytic differentiation (5, 6), and in PC12 cells intranuclear translocation of the subunit from cytosol to the nucleus was observed upon the stimulation of cells with nerve growth factor (30). In osteosarcoma Saos-2 cells the subunit is distributed not only in cytosol but also in the nucleoplasm and nucleoli, and after the stimulation of cells with interleukin 1 it also translo-cates from cytosol to the nucleus (31). Sonification and centrifu-gation of the intact liver nuclei may lead to the distribution of enzyme in both the soluble fraction and nuclear membranes (4). Using a different approach (preparation of membrane-depleted nuclei by detergent), the present study confirms the 3-kinase activity in the nuclear envelope observed above but also shows that within the intranuclear region there is no such activity, because there was no radiolabeling of PtdIns(3,4,5)P 3 even when the exogenous lipid substrate (PtdIns(4,5)P 2) was added. Furthermore, following partial hepatectomy no increase in the above-mentioned 3-kinase activity in the nuclei could be observed, suggesting that class I PI3Ks is not involved in nuclear signaling during compensatory liver growth.

In contrast to class I PI3Ks, which are mainly cytosolic and are subjected to translocation to the membranes upon cell stimulation (Ref. 32 for review), class II PI3Ks are predomin-antly associated with membrane fractions of cells (28, 33) and, therefore, are good candidates for compartmentalization within the cell nucleus as has been shown for PtdIns 4-kinase and PtdIns(4)P 5-kinase (34). Indeed, from the present study it can be concluded that PI3K-C2β is not present in the nuclear envelope and is probably attached to the nuclear matrix as has been shown for the above-mentioned kinases (34). It is impor-tant to note that polyclonal antisera against PI3K-C2β could not be used for immunohistochemical studies2; therefore, no exact localization of PI3K-C2β in the membrane-depleted cell nuclei could be done. Nevertheless, knowing that in the membrane-depleted nuclei the concentration of PtdIns is about 20 times higher than PtdIns(4)P (23) and that in in vitro conditions with equimolar concentration of substrates, PtdIns(4)P could be phosphorylated only in the presence of Mg 2+ with substantially less efficiency than PtdIns(present study), it seems obvious that in vivo PI3K-C2β phosphorylates PtdIns to produce PtdIns(3)P, which has also been observed with purified recombinant enzyme (28).

Interestingly, the increased activation of PI3K-C2β and the formation of PtdIns(3)P parallels the increase in nuclear PLC activity, DAG concentration, and translocation of PKC to the nucleus following partial hepatectomy (23, 29). This indicates that changes in the nuclear inositol lipid metabolism precede the S-phase of cell cycle and mitosis, because cells reach the peak of proliferation 22–26 h after partial hepatectomy (35). Although potential targets for nuclear PKC include lamins, DNA polymerase, and topoisomerase II (see Refs. 3, 36–38 for reviews), one can only speculate about the function of PtdIns(3)P in the cell nuclei. The observations that negatively charged phospholipids can stimulate RNA synthesis by affect-ing chromatin organization (39) and that histones H1 and H3 are PtdIns(4,5)P 2-binding proteins (40) indicated the impor-tance of nuclear polyphosphoinositides in transcription events. On the other hand, intranuclear PtdIns(3)P may be involved in trafficking events, as has been shown for vesicle trafficking via its interaction with FYVE finger domain (11, 32).

In platelets, PI3K-C2β is activated following stimulation of the integrin receptor with fibrinogen, and this activation could be prevented by calpain inhibitors (17). The present study extends this observation by showing that short term exposure of enzyme to calpain resulted in a similar degree of activation compared to the nuclei harvested 20 h after partial hepatec-tomy, suggesting that calpain-mediated proteolysis of the en-zyme may be responsible for its activation. The calpains are predominantly cytoplasmic Ca 2+-dependent proteases, and there are two well-characterized calpain isozymes: μ-calpain shows proteolytic activity at μM Ca 2+ concentrations, whereas μ-calpain is already active at micromolar Ca 2+ concentration (41). Nevertheless, both isoforms are likely to be able to process cellular substrates at physiological Ca 2+ concentrations (42). It is important to note that in the isolated liver nuclei some high molecular mass (120–200 kDa) matrix proteins were sub-strates for purified calpains (43), which, together with the observation that μ-calpain is transported into cell nuclei in an

2 S. Volinia and H. Bansić, unpublished observation.
ATP-dependent fashion (44) and accumulated evidence that there is calcium signaling in the cell nucleus (45, 46), strongly supports the present evidence for calpain-mediated activation of PI3K-C2β in the nuclei when the cells are subjected to growth and hyperplasia. It is noteworthy that the deletion of C2 domain of PI3K-C2β increased the lipid kinase activity (28), and from an amino acid sequence of the enzyme it could be deduced that calpain-mediated proteolysis may cleave C2 domain, which may result in the observed gel shift and the activation of the enzyme. On the other hand, rapid recruitment of PI3K-C2β to a phosphotyrosine-signaling complex containing epidermal growth factor receptor (18) suggests that there are different mechanisms of enzyme activation, which depend on the activation of different cellular receptors and/or different subcellular localization of the enzyme.

In summary, the data presented in this report show that, in the membrane-depleted nuclei during the compensatory liver growth, there is an increase in PtdIns(3)P formation as a result of PI3K-C2β activation. Addition of exogenous μ-calpain to native membrane-depleted nuclei resulted in a similar degree of PtdIns(3)P formation strongly suggesting that PI3K-C2β activation may be a calpain-mediated event.

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