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# Nuclear inositides*

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## ABBREVIATIONS

DAG, 1,2-diacylglycerol; EGF, epidermal growth factor; TNF, tumour necrosis factor; IGF-I, insulin-like growth factor I; Ins(*n*)P, inositol phosphate; Ins(*n*,*n*)$P_2$, inositol bisphosphate; Ins(*n*,*n*,*n*)$P_3$, inositol trisphosphate; MEL, murine erythroleukemia (Friend); PIC, phosphoinositidase C (phosphatidylinositol-specific phospholipase C); PKC, protein kinase C; PKB, protein kinase B; PLC, phospholipase C; PLD, phospholipase D; PMA, phorbol 12-myristate 13-acetate; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdIns, phosphatidylinositol; PtdIns(x)P, phosphatidylinositol monophosphate, phosphorylated at position x; PtdIns(*x*,*y*)$P_2$, phosphatidylinositol bisphosphate, phosphorylated at position x and y; PtdIns(*n*,*n*,*n*)$P_3$, phosphatidylinositol trisphosphate; PtdOH, phosphatidic acid.

## INOSITOL LIPIDS

Inositol lipids are made up of an inositol head group that is linked, via a phosphate di-ester bond to a DAG moiety. The DAG acts to maintain this lipid as part of the membrane while the inositol head group forms a potential interface between components in the cytosol and the membrane. PtdIns, the precursor for all signaling inositol lipids, can be phosphorylated in a number of positions, by a number of different kinases to yield at least seven potential second messengers (see Fig. 1) which act in two ways. Some of these can be cleaved by a specific phospholipase to yield both a membrane bound and a soluble cytosolic second messenger. This has been shown to be the case for the hydrolysis of PtdIns(4,5)P2 by PIC to generate DAG, which acts to stimulate PKC (Nishizuka and Nakamura, 1995), and Ins(1,4,5)P3 which regulates calcium release from internal stores(Berridge and Irvine, 1984; Streb *et al.* 1983). In this case PKC acts as a sensor which integrates these two signals, leading to the phosphorylation of key cellular components (Oancea and Meyer, 1998). [The mechanism by which DAG activates PKC is thought to involve binding to the membrane, followed by a conformational change which leads to an opening of the active site, and then activation of the enzyme.] Thus this lipid acts not only to localise, but also plays a key role in its activation. This idea now appears to be a recurring phenomenon, as a number of phosphorylated forms of inositol lipids have also been shown to bind, and therefore specifically localise key signaling enzymes. One of the first examples of this paradigm is the activation of PKB (or AKT). This protein kinase contains a pleckstrin homology (PH) domain, examples of which have been shown to be important in the interaction with proteins, such as the βγ subunits of heterotrimeric G proteins, or with lipid components of the membrane (Bottomley *et al.* 1998; Harlan *et al.* 1994; Rameh *et al.* 1997; Tanaka *et al.* 1997; Lin *et al.* 1997; Klarlund *et al.* 1998; Fushman *et al.* 1998).

That inositol lipids are able to target proteins and lead to their activation is now an established role

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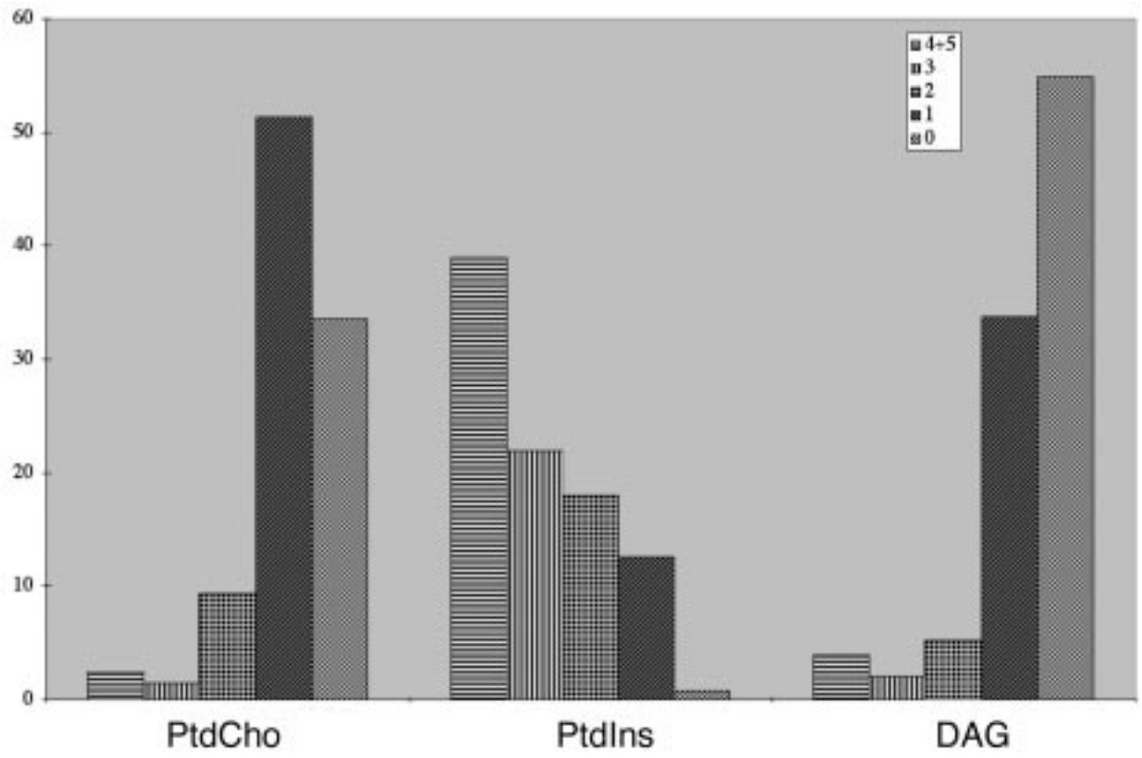


**Fig. 1 -** Nuclear PtdCho or PtdIns was hydrolysed using specific phospholipases. The DAG was isolated and phosphorylated using a non-specific DAG-kinase. The products were dimethylated using diazomethane and separated on silver coated plates (5%). The unsaturated lipids interact with the silver, with the strength of the interaction depending on the number of double bonds. Thus DAGs containing saturated fatty acids will migrate to the top of the plate, whereas those with arachidonyl (4 double bonds ) will only migrate a short distance. As can be seen the DAG backbone of PtdCho is predominantly disaturated and monounsaturated (>80%) while those from PtdIns contain mainly 3,4 and 5 double bonds (>80%). The nuclear DAG was found to be predominantly made up of DAG with disaturated and monounsaturated backbones, suggesting that they could not be derived solely from PtdIns hydrolysis.

for these lipids, and it is likely that the different lipids will utilise different binding domains. There are now seven different phosphorylated forms of PtdIns which are present in cells, and together with the fact that they are likely to be located in various intracellular compartments this would provide a network of target sites able to activate and localise proteins important in the regulation of a number of key pathways within the cell.

## INOSITIDES IN THE NUCLEUS

Although the role of these lipids in the cytosolic compartment of a cell is well established, their role in the nucleus has generated a certain amount of controversy. However, the availability of cDNAs and antibody probes to the enzymes that modify these lipids (Katan, 1998; Balla, 1998; Hinchliffe *et al.* 1998) and their unequivocal nuclear localisation should finally put the issue of contamination to rest, and open up new investigations into putative nuclear targets.

Smith and Wells (Smith and Wells, 1983b; Smith and Wells, 1984b; Smith and Wells, 1983b) were the first to demonstrate that, when purified intact rat liver nuclei were incubated with 32P-ATP, PtdOH, PtdIns(4)P and PtdIns(4,5)P2 were rapidly labeled. The interpretation of this is that the enzymes that phosphorylate DAG, PtdIns and PtdIns(4)P, together with their substrates, are present in nuclei. As this experiment uses endogenous substrates it also suggests, importantly, that the enzymes and the lipids that they phosphorylate are

present in the same location. This type of experiment has been carried out using nuclei from various tissues and cell types with results that are essentially the same as the original data from Smith and Wells (Divecha *et al.* 1993a; Billi *et al.* 1993a; Santi *et al.* 1992a; Miscia *et al.* 1991a; Cocco *et al.* 1988a; Cocco *et al.* 1987a; Cocco *et al.* 1987a; Vann *et al.* 1997a). A number of studies have taken these experiments further, in order to define whether this nuclear pathway is a target of regulation by growth factors and cellular processes (Miscia *et al.* 1991; Rana *et al.* 1994; Cataldi *et al.* 1994; Cataldi *et al.* 1990; Cocco *et al.* 1988; Cocco *et al.* 1987; Banfic *et al.* 1993; Divecha *et al.* 1995; Divecha *et al.* 1993). Nuclei isolated from control MEL cells, or from those differentiated down an erythroid pathway, showed differences in their labeling patterns after incubation with 32P-ATP. Specifically the amount of label incorporated into PtdIns(4,5)P2 was substantially increased after differentiation(Billi *et al.* 1993; Cocco *et al.* 1987). This may have been due to changes in the inositol lipid modifying enzymes (either kinases/phosphatases or phospholipases) or in the levels of their substrates, or both. This experiment, however, led to the hypothesis that this nuclear inositide cycle could be regulated distinctly from that of the plasma membrane. Further, when quiescent Swiss 3T3 cells were stimulated with IGF-1, changes in the nuclear inositides were seen, with no such changes in the whole cells (Cocco *et al.* 1988). These changes were shown to reflect mass levels of the various inositides and the simplest interpretation of the data was that IGF-1 led to an increase in the activity of a nuclear PIC, which hydrolysed either PtdIns(4)P or PtdIns(4,5)P2, leading to enhanced production of nuclear DAG (Divecha *et al.* 1991; Cocco *et al.* 1989). This increase in nuclear DAG occurred concomitantly with translocation of PKC to the nucleus, a physiological downstream target of this lipid (Neri *et al.* 1994; Martelli *et al.* 1991; Martelli *et al.* 1991; Divecha *et al.* 1991). Indeed, IGF-1 was shown to enhance the activity of a nuclear PIC whilst having no affect on its cytosolic counterpart (Martelli *et al.* 1999; Martelli *et al.* 1992). Studies either on differentiating cells (D'Santos *et al.* 1999; Divecha *et al.* 1995; Martelli *et al.* 1994) or on proliferating tissue, e.g. partial hepatectomy (Liu *et al.* 1996; Kuriki *et al.* 1992; Banfic *et al.* 1993), and their effects on the nuclear inositide cycle, have led to the suggestion that regulation of a nuclear PIC may be a key event during proliferation and terminal differentiation. Thus a nuclear PIC is able to regulate the mass levels of nuclear DAG which in turn regulates progression through the cell cycle, perhaps through regulation of PKC activity. Extension of this hypothesis would suggest that improper control of the levels of nuclear DAG should lead to problems with progression through the cell cycle. Indeed, studies carried out by Topham et al (Topham *et al.* 1998; Ding *et al.* 1997) demonstrated that DAG kinase ζ, which is partially nuclear localised, is phosphorylated after stimulation of PKC, by EGF or TPA, and this leads to its efflux from the nucleus. This efflux appears to correlate with an increase in the mass of nuclear DAG. The PKC phosphorylation site was mapped to a putative nuclear localisation sequence, which, when phosphorylated, prevents the enzyme from entering the nucleus. In support of the above hypothesis, overexpression of this enzyme led to a decrease in the nuclear DAG and a doubling in the cell cycle time, with cells becoming blocked in G1. Interestingly, these effects were dependent on both the DAG kinase activity and its nuclear localisation.

## NUCLEAR DAG IN MEL CELLS IS NOT GENERATED THROUGH THE HYDROLYSIS OF PTDINS DERIVED LIPIDS

Stimulation with growth factors or proliferation (induced by partial hepatectomy) leads to an increase in the levels of nuclear DAG, which is correlated with an increase in nuclear PIC activity (Banfic *et al.* 1993). In contrast to this, when MEL cells were terminally differentiated, leading to a cell cycle arrest, a decrease in the level of nuclear DAG was observed, which correlated with a down-regulation of a nuclear PIC activity (Martelli *et al.* 1994; Billi *et al.* 1993; Cocco *et al.* 1995; D'Santos *et al.* 1998; Divecha *et al.* 1995). More specifically, differentiation led to a decrease in the transcription of the gene encoding PLCβ1 (Martelli *et al.* 1994). We and others have demonstrated that this was potentially the nuclear PIC activity, as this was the only immunologically detectable isoform of PIC in rat liver nuclei (Divecha *et al.* 1993b; Divecha *et al.* 1995b; Martelli

*et al.* 1995b; Zini *et al.* 1993b; Martelli *et al.* 1992b). Thus the increase/decrease in nuclear DAG resulting from different treatments has been ascribed to the changes in this isoform of PIC. Using a beautiful technique set up by Kennerly, we tried to establish the identity of the precursor lipid for the nuclear DAG, with the assumption that it would be derived from hydrolysis of a PtdIns lipid. The technique is well suited to analysis of nuclear lipids as it does not depend on isolation of mass quantities of lipid, but is based on the analysis of PtdOH after separation according to the number of double bonds they contain i.e. their degree of saturation. PtdIns is predominantly polyunsaturated, while the PtdCho is predominantly saturated or monounsaturated. PtdIns and PtdCho were hydrolysed using specific PLCs and their respective DAGs were phosphorylated with a non-specific DAG kinase, to generate labeled PtdOH. These were then separated by argentation chromatography, which distinguishes molecular species by their degree of saturation. These patterns of unsaturation were then compared to the overall nuclear DAG pool after phosphorylation to PtdOH by the same DAG kinase (Fig 1.)

The result demonstrated that the majority of nuclear DAG **could not** be derived from the hydrolysis of PtdIns and must either come from the *de novo* pathway, or be derived from the hydrolysis of other lipids such as PtdCho (D'Santos *et al.* 1999). Upon differentiation of these cells, which was shown to lead to a decrease in nuclear PIC activity, the decrease in nuclear DAG occurred in species that are already present in control nuclei and therefore unlikely to be derived from PICβ1mediated cleavage of PtdIns lipids (data not shown). Indeed we generated a stable MEL cell line that constitutively overexpressed PLCβ1 from a CMV promoter (Fig. 2). Analysis by western blotting after differentiation showed that the enzyme was no longer downregulated. Analysis demonstrated that these cells still underwent differentiation, as assessed by hemoglobin production, and also were terminally differentiated as shown by a G1 cell cycle blockade (data not shown). However, we were still able to show that there was a decrease in the levels of nuclear DAG suggesting that PICβ1 is not required for the generation of nuclear DAG.

As previous data from ours and others labs have demonstrated that a nuclear PIC is present and is able to hydrolyse phosphoinositides, we sought to determine if PtdIns hydrolysis was actually occurring in the context of an intact nuclei. After in vitro labeling of nuclei with 32P-ATP the radioactive phosphatidic acid was analysed using the above method to separate different molecular species.

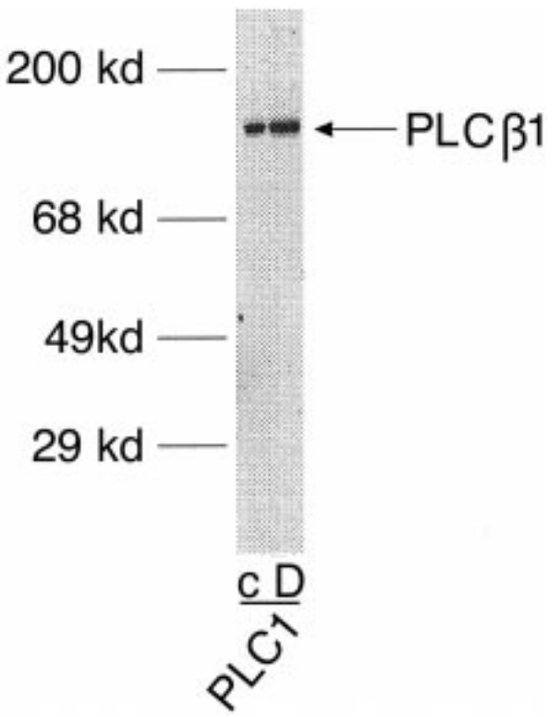


**Fig. 2 -** Cell lines were derived after transfection of the MEL cells with myc-PICβ1 under the control of a CMV promoter. After selection and single cell cloning by limiting dilution, cells were expanded and either treated as controls or differentiated using 1.5%DMSO. Cell lysates were then blotted for PICβ1 using either and antibody against this isoform (data not shown) or with an antibody specific to the Myc tag. As can be seen the enzyme is no longer downregulated after differentiation. Analysis using an assay for PIC activity demonstrated that there was 5 times more PIC activity associated with the nuclear fraction in overexpressing cell lines compared to the parental line, which was not downregulated during differentiation. Hemoglobin analysis demonstrated that PIC β1 overexpressing cells still differentiated and also cell cycle analysis showed that they still blocked in G1 of the cell cycle. Analysis of nuclear DAG showed that the decrease was still observed. The initial observation that PtdIns(4,5)P2 levels are increased after differentiation and that this was due to a decrease hydrolytic activity associated with the nucleus was also not abrogated in cell lines expressing the PICβ1. These data suggest that PIC β1 is not the PIC activity that generates nuclear DAG and that downregulation of this enzyme is not a prerequisite for differentiation.

What became apparent is that the phosphatidic acid was predominantly poly-unsaturated, having a diacylglycerol backbone similar to PtdIns derived lipids. This suggested that these DAGs are directly phosphorylated to PtdOH. To illustrate this further we showed that two structurally different phospholipase inhibitors were able to block the production of labeled PtdOH during an in vitro labeling reaction and that this inhibition occurred in the species that were similar to the DAG which would be derived from the hydrolysis of PtdIns lipids (Fig. 3). No such inhibition was found with an inactive PLC inhibitor or with inhibitors of either PLD (butanol) or of a PtdCho specific PLC (D609).

Thus these data would suggest that there are at least two pools of nuclear DAG. A minor pool which is generated through the action of a PIC activity and a major pool which is generated in a different manner, but reflect the DAG backbone of PtdCho. Previous data in 11c9 cells also suggested that thrombin stimulation led to an increase in nuclear DAG mass, which was most likely derived from PtdCho hydrolysis (Leach *et al.* 1992; Jarpe *et al.* 1994). We have carried out this analysis in a number of different cell types (including Swiss 3T3 cells) and always find that the majority of DAG resembles PtdCho rather than PtdIns. How nuclear DAG is generated and whether nuclei contain a PtdCho specific PLC is suggested, but not demonstrated. This also raises the interesting question over which species of DAG are involved in the regulation of cell cycle progression. In the

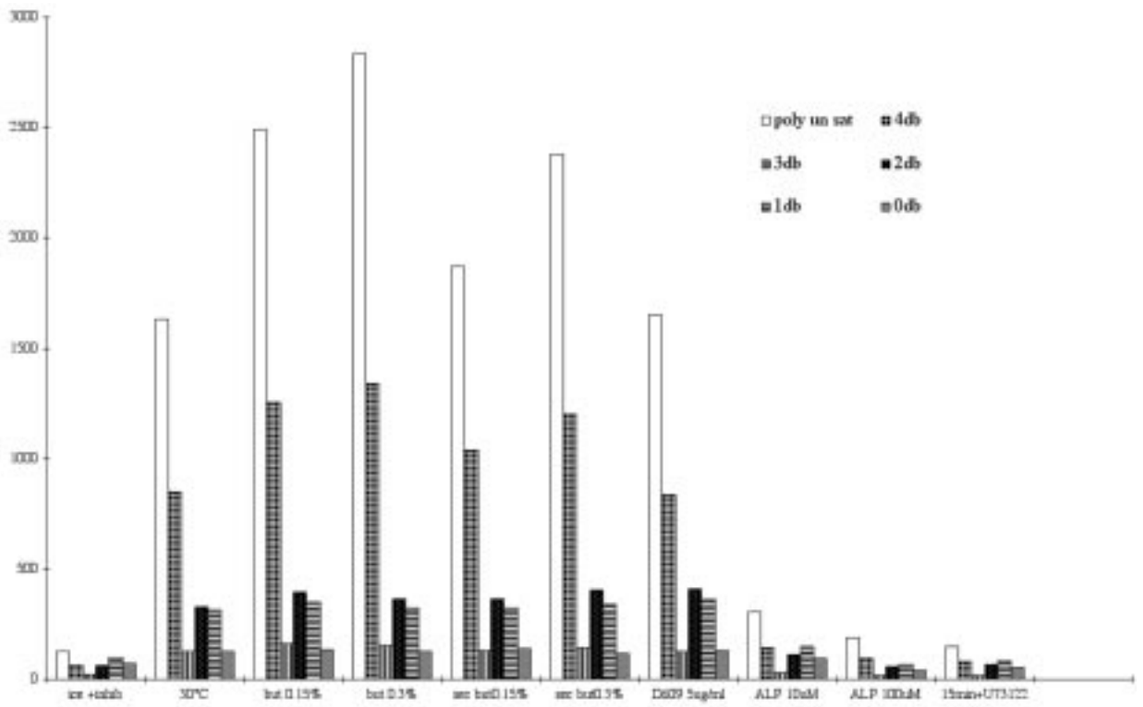


**Fig. 3 -** Intact nuclei were incubated either at 30 or on ice for 5 minutes in the presence or the absence of the indicated inhibitors. The PtdOH was then labeled by the addition of 32P-ATP for a further 2.5 minutes. In the case of the ice control incubation was carried out on ice in the presence of the inhibitor the transferred to 30oC for labeling. Control experiments were carried out whereby nuclei were incubated for 5 minutes first to generate DAG in the absence of ATP. The inhibitor was then added and the labeling carried out for a further 5 minutes. The PtdOH was then dimethylated and the various DAG backbones were separated using argentation chromatography. The inset is the legend for the number of double bonds. These data indicated that the inhibitors did not inhibit the nuclear DAG kinase which was able to phosphorylate the DAG generated through PtdIns-hydrolysis even in the presence of the inhibitor. The data suggest that DAG generated by PtdIns hydrolysis is a substrate for the nuclear DAG kinase. Further experiments using exogenous DAG suggested that this enzyme was not specific for these DAGs, but rather the enzyme was probably located such that it was only able to phosphorylate this DAG.

study carried out by Topham *et al.* (Topham *et al.* 1998) overexpression of nuclear DAG kinase, leading to the decrease in nuclear DAG content, led to a cell cycle block. Which types of DAG are required for cell cycle progression is not known, but it is important to ascertain this as it defines which type of PLC is regulated during the cell cycle. Our data on changes in the cell cycle DAG would suggest that the major pool of DAG does not change during cell cycle progression. We were, however able to demonstrate that the PtdOH derived in an *in vitro* reaction did change, suggesting an apparent activation of a PIC activity as cell progressed through the G1/S boundary. Further work is required, however to characterise these changes and to determine which isoform of PIC is activated and which phosphoinositides serve as the substrate. This point is important as it determines which head group is released. In light of the contentious issue surrounding the presence of the Ins(1,4,5)P3 receptor on the inner nuclear membrane and the specific regulation of nuclear calcium, only hydrolysis of PtdIns(4,5)P2 would generate this head group (see PtdIns(4,5)P2). It is also not clear if the role of the PIC mediated hydrolysis is to generate new second messengers such as DAG, Ins(1,4,5)P3 and PtdOH, or to attenuate the signaling capacity of PtdIns(4,5)P2. Which PIC is acting within the nucleus? There is evidence for the presence of a number of different PIC isoforms present within the nucleus (D'Santos *et al.* 1998)and this may reflect the various cells types that have been studied or may reflect the need for different PICs in the regulation of multiple pools of nuclear PtdIns(4,5)P2.

## THE SYNTHESIS OF NUCLEAR PtdIns(4,5)P2

Potentially PtdIns(4,5)P2 could represent a signaling molecule within the nucleus. Its levels could be regulated by either its synthesis and/or by its hydrolysis. The demonstration that both the type 1 and the type 11 PIPkins are present in nuclei would suggest that this lipid is regulated in a number of different ways. PtdIns(4,5)P2 within the nuclear matrix is probably organised such that its hydrophobic tail is bound to some protein, while its head group is able to sit out into the nucleosol. This would make it ideal for use as a targeting agent, as a recent study has suggested (Zhao *et al.* 1998). Stimulation of immature T cells leads to their movement from G0 into G1 of the cell cycle. This traversal is accompanied with a decondensation of chromatin and an increase in nuclear size (these two events are thought to be important in the regulation of transcription and for DNA synthesis to occur). One of the nuclear complexes thought to be important is the BAF complex. This is a large complex of at least thirteen proteins which, on binding to DNA and the nuclear matrix, are thought to be important in its remodelling. On stimulation of the T cells there is an increase in the amount of BAF complex that becomes associated with the nuclear matrix. Interestingly, the ability of this complex to associate with these nuclei is PtdIns(4,5)P2 dependent (Zhao *et al.* 1998). However, no data has yet demonstrated an increase in the amount of PtdIns(4,5)P2 during this process of maturation or that this binding is specific to PtdIns(4,5)P2 (over that obtained with PtdIns(4)P, PtdIns(3,5)P2 or PtdIns(3,4)P2). It is, however, possible that stimulation leads to the regulation of a PIPkinase, which leads to an increase in the nuclear PtdIns(4,5)P2 levels, and that this is important in the translocation and regulation of the BAF complex. Interestingly, previous data has suggested that the addition of lipids, such as PtdIns or PtdCho could lead to profound affects on the transcription, *in vitro*, of a number of genes through their affects on chromatin structure (Manzoli *et al.* 1975; Cocco *et al.* 1976; Manzoli *et al.* 1977; Manzoli *et al.* 1978; Maraldi *et al.* 1984; Cocco *et al.* 1985; Capitani *et al.* 1986).

PtdIns(4,5)P2 can also bind specifically to histones H1 and H3 and this binding is able to inhibit the histone-mediated repression of RNA-polymerase 1, leading to enhanced *in vitro* transcription, (Yu *et al.* 1998) and has also been suggested to be important in the regulation of RNA efflux, by its interaction and activation of a nuclear envelope associated ATPase (Smith and Wells, 1984b). More recent data has suggested that the type 11 PIPkinases may play a role in the regulation of mRNA splicing, as immunofluorescence showed a colocalisation of this enzyme with RNA splicing complexes within the nucleus (Boronenkov *et al.* 1998). These authors were also able to show a partial colocalisation with nuclear PtdIns(4,5)P2 using antibodies specific for this lipid. Thus PtdIns(4,5)P2 may play a role in

chromatin remodelling during a number of different key nuclear processes, such as transcription, DNA synthesis and during the condensation/decondensation of chromatin during mitosis. That PtdIns(4,5)P2 may play such diverse roles in the nucleus would suggest that its synthesis would be tightly regulated and that there would be multiple mechanisms for the regulation of this process. In the cytosol there are at least two distinct PtdInsP kinases that are able to synthesis this lipid. The first is a bone fide PtdIns-(4)P 5-kinase, or type 1 PIPkin, which phosphorylates on the 5 position to generate PtdIns(4,5)P2. Thus far there are three subtypes of this family, $\alpha$, $\beta$, and $\gamma$ isoforms which are probably regulated in different ways. This family of enzymes has been shown to be regulated by a number of small molecular weight g proteins of the rho family such as rho and rac, but also recent data suggests that the ARF family are also potent activators of this enzyme, further evidence that PtdIns(4,5)P2 is involved in both cytoskeletal dynamics and in the regulation of vesicle trafficking. In the nucleus of MEL cells we have demonstrated that a type 1 pipkinase is also present, which is specific for phosphorylation of the 5 position of PtdIns(4)P. Our data also suggests that this enzyme may be a target for regulation by DNA damage agents, as in vitro labeling assays after treatment with Cisplatin lead to a specific increase in the synthesis of PtdIns(4,5)P2. This occurred through the activation of an activity that predominantly phosphorylated PtdIns(4)P on the 5-position. How this enzyme is regulated, or which isoform it is, is not known, although our data using isoform specific antibodies in MEL cell nuclei would suggest that it is a murine-type 1 $\alpha$.

Recent data has suggested that nuclei also contain a type 11 enzyme. This enzyme is able to phosphorylate the 4-position of PtdIns(5)P to also generate PtdIns(4,5)P2. The presence of this enzyme in the nucleus would also suggest that regulation of PtdIns(4,5)P2 levels in the nucleus is complex. Immunoprecipitation using a type 11 specific monoclonal antibody demonstrated that MEL cell nuclei also contain a type 11 enzyme. We were therefore interested to assess if in intact nuclei this enzyme was able to generate PtdIns(4,5)P2. To investigate this nuclei were labeled for 2.5 minutes with 32P-ATP and the PtdIns(4,5)P2 was isolated after TLC. To determine if any labeling had occurred in the 4 position we dephosphorylated this PtdIns(4,5)P2 with a specific 5-phosphatase. The products of this reaction were then separated again using TLC and the counts present in the 4 position were determined by quantitation of the PtdIns(4)P levels. The number of counts present in the 5-position was determined by the decrease in the counts in PtdIns(4,5)P2. We found that there was a ratio of about 5:1 in terms of the 5:4 phosphate labeling, suggesting indeed that some 4-phosphorylation was occurring in the nucleus. This could, however come from the 4 phosphorylation of PtdIns, which was then phosphorylated on the 5-position by a PtdIns(4)P-5-kinase. The ratio of labeling would then just reflect the size of the cold pools of PtdIns(4)P. However, for type II enzyme to be active would require that its substrate was also present, within the nucleus. Using recombinant type I and type II enzymes we have established a mass assay for both PtdIns(4)P and for PtdIns(5)P. These assays are specific and the enzymes under the conditions used show no cross specificity. Using these assays we have shown that MEL cell nuclei contain approximately 5% PtdIns(5)P and 5%PtdIns(3)P with the remaining 90% consisting of PtdIns(4)P. The issue of how PtdIns(5)P is generated is still contentious as it is not clear if it comes from the breakdown of PtdIns(4,5)P2, by a specific 4 phosphatase, or if there is a PtdIns-5-kinase. Recent data demonstrated that a mammalian homologue of the yeast type III PIPkin shows specificity for phosphorylation of PtdIns on the 5-position, suggesting that this may be the elusive PtdIns 5-kinase. To determine if nuclei contain a PtdIns-5kinase, we isolated the labeled PtdInsP after in vitro labeling and phosphorylated this with either the type I or type II enzymes in the presence of cold ATP. The data revealed that these nuclei contain both a PtdIns-4 and a PtdIns-5-kinase and also a PtdIns-3-kinase. Although these data still suffer from the fact that this could be derived from the dephosphorylation of PtdIns(4,5)P2. However in the time scale of the assay this is unlikely. Preliminary data suggest that fractions isolated from chromatography of MEL cell nuclear lysates on a heparin column have a kinase that is also able to generate PtdIns(5)P.

## CONCLUSIONS

It now appears that inositol lipid metabolism within the nucleus is as complex as that within the cytosol. There are both lipid kinases to generate

all of the known inositol lipids shown to be present in the cytoplasm as well as a number of phosphatases able to degrade them. Also most of the phospholipase C and D that are able to hydrolyse PtdIns or PtdCho respectively have also been shown to be present within the nucleus. Thus the generation of nuclear DAG also appears to be complex with multiple modes of regulation. Which of the inositides is hydrolysed within the nucleus is still unclear as is which pool of DAG is important in the activation of nuclear PKC. The demonstration that nuclear DAG derived from PtdIns hydrolysis is phosphorylated directly to generate PtdOH may suggest the synthesis of another potential second messenger. The role of PtdOH as a second messenger appears to be firmly accepted, however there are as yet no real targets for this lipid. Isolation using affinity columns, as has been used in the isolation of specific PtdIns(3,4,5)P3 binding proteins, should yield new targets. Methods of analysing specific PtdOH formation within living cells, would then be a real possibility.

It is also clear that there are present in nuclei multiple mechanisms for regulating PtdIns(4,5)P2 levels, but the pathways that impinge on this synthesis are still poorly understood. Data presented at this meeting from Lucio Cocco and Alberto Martelli laboratories would suggest that PICβ1 is a key phospholipase within the nucleus, and that it may be regulated by the translocation of MAP-kinase into the nucleus induced by growth factor activation. However there are several other reasons why a cell may want a signaling system within the nucleus. Just as cells have to respond to external environmental stimuli, such as growth factors, the cell must also signal from the nucleus to the rest of the cells. Conditions that lead to DNA damage or to the inactivation of DNA synthesis lead to the activation of signaling cascades which lead to a cell cycle arrest. Thus the cell requires mechanisms to sense these changes and then to respond by upregulating certain signals. Cells then make decisions whether to continue (if the damage is repairable) or to move into apoptosis. The lipid PtdIns(3,4,5)P3 is at the middle of these decisions as it can regulate the activity of PKB, a protein that is crucial for cell survival. This protein also functions within the nucleus, although what its role is, and how it is regulated there is at this moment unclear. However, the nuclear inositide pathway may represent a signaling pathway that is regulated by these nuclear stimuli. Further work to isolate specific nuclear enzymes and charactersise how these are regulated with respect to cell cycle progression and these internal stimuli will yield new targets for potential therapies.

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