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CHAPTER 5

Phosphoinositide signalling in the nucleus

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Introduction
Phosphorylation at the 3, 4, or 5, position of the inositol head group of phosphatidylinositol generates seven different phosphoinositides that form the basis of a ubiquitous membrane signalling system. An array of tightly regulated phosphoinositide kinases and phosphatases, ultimately control the subcellular profile of phosphoinositides (Irvine, 2005). Phosphoinositides can regulate protein localisation, ion channel function and protein enzymatic activity, which can impact on cellular processes including vesicle transport, cytoskeletal dynamics, cell proliferation and survival, gene transcription, cell polarity and migration (McCrea and DeCamilli, 2009). Phosphoinositides are tethered tightly into the membrane and can recruit and localise proteins to specific subcellular membrane domains through specific phosphoinositide interacting domains (PID) (Lemmon, 2003). Because the membrane can be considered more akin to a two dimensional system, membrane interaction is analogous to inducing protein/protein interactions and acts to concentrate upstream regulators and downstream targets together leading to enhanced downstream signalling and specificity. Phosphoinositide signalling occurs on many different intracellular membranes including the inner surface of the plasma membrane, the golgi, the endoplasmic reticulum and on membrane vesicles that move between these compartments and their deregulation had been implicated in an array of human diseases (McCrea and DeCamilli, 2009).

A more quirky aspect of phosphoinositide signalling occurs within the nucleus, which will be the subject of this review. This review is rather a personal view of phosphoinositide signalling in the nucleus and for a more comprehensive review of the literature the reader is directed to the following (Irvine, 2006; Gonzales and Anderson, 2006).

What is quirky about nuclear phosphoinositide signalling?
Phosphoinositides are normally present within the context of a membrane. Phosphoinositides contain two long hydrophobic fatty acyl tails linked to a glycerol group, which is coupled via a phosphodiester linkage to the phosphorylated inositol head group. This chemical structure is ideally suited to form the interface between the hydrophobic membrane, through insertion of the fatty acyl tails, and the cytosol. The nucleus is an organelle that is bounded by a double bilayer membrane, the outer part being contiguous with the endoplasmic reticulum. Sense would postulate that inositide signalling in the nucleus might occur on the inner surface of this double bilayer. The first clue that this may not be the case came from studies in MEL cells in which Cocco and colleagues (Cocco et al., 1987) first demonstrated that changes in nuclear inositides could occur independently of those in the plasma membrane. In this case nuclei were isolated from control MEL cells or from cells that had been
differentiated down the erythrocyte pathway. The isolated nuclei were then incubated with radiolabelled ATP, which became incorporated into phosphoinositides. As the nuclei were intact and not disrupted, as assessed by electron microscopy, these data showed that phosphoinositides were present in nuclei and that the kinases that can make them are also present and must be located at the same sites. What was fascinating and previously undocumented, was that upon differentiation there were changes in the amount of radiolabelled phosphoinositide present in the nuclei. This suggested that phosphoinositides are dynamically regulated in response to extracellular signals and that phosphoinositides in the nucleus may constitute a signalling pathway that could specifically control nuclear functions. The rest is history and since then a complex phosphoinositide signalling system has been uncovered in the nucleus, the major aspects of which are outlined in Fig. 1.

Figure 1. Phosphoinositide pathways and downstream targets in the nucleus. The figure shows the major phosphoinositide pathways that are present in the nucleus with some of the downstream targets (dark blue) and nuclear functions (green) that are regulated. Second messengers are outlined in red boxes. (for interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

So far so good! Nothing too quirky about this pathway yet! The surprise came when the same researchers isolated the nuclei in the presence of detergents. The chemical nature of detergents means that they are extremely good at solubilising membrane structures. In fact, if you did a similar experiment with isolated plasma membranes there would be no phosphoinositides left. However, isolation of nuclei in the presence...
of detergents did not prevent either the radiolabelling of nuclear phosphoinositides in the control conditions nor did it remove the changes in phosphoinositide labelling observed upon differentiation. What the detergents did remove, however, was the double bilayer membrane as assessed by electron microscopy (Cocco et al., 1987). In a more detailed analysis, we prepared nuclei from rat liver, which have a beautiful intact nuclear envelope after isolation, and used increasing concentrations of detergent to remove the envelope, which was analysed by electron microscopy. Radiolabelling of nuclear phosphoinositides and the mass of various phosphoinositides and phospholipids were also measured (Vann et al., 1997). The data clearly demonstrated that removal of the nuclear envelope correlated with loss of phospholipids such as phosphatidylcholine, but did not correlate with either the removal of phosphoinositides or phosphoinositide kinases. In a different study on the dynamic changes in nuclear phosphoinositides induced by IGF-1 treatment, we also found that in fact it was important to remove the nuclear envelope in order to be able to monitor changes in the nuclear signalling phosphoinositides (Divecha et al., 1991). These data together with other studies suggest that the phosphoinositide pools that can be regulated and are therefore likely involved in regulating nuclear processes, are present within the nucleus rather than in the nuclear envelope (Divecha et al., 1991; Banfic et al., 1993; Cocco et al., 1987, 1988; Payrastre et al., 1992). However, and this is where nuclear phosphoinositide signalling becomes quirky, using electron microscopy no internal membrane structures have been found within the nucleus. So where are these phosphoinositides localised and how are they maintained there? Using a specific PID or antibodies that interact specifically with the phosphoinositide phosphatidylinositol(4,5)bisphosphate \( \text{PtdIns}(4,5)P_2 \), it appears that \( \text{PtdIns}(4,5)P_2 \) and by inference other phosphoinositides, is clustered in nuclear structures called interchromatin granules (Watt et al., 2002; Boronenkov et al., 1998; Mellman et al., 2008). These structures are also nuclear regions that are highly enriched in factors used for splicing mRNA. Although this would suggest a role for \( \text{PtdIns}(4,5)P_2 \) in splicing it not clear whether these regions are where splicing occurs or if they are storage compartments for splicing components. The exact chemical nature of phosphoinositides in these structures is far from clear. Because of the fatty acyl tail it is unlikely that phosphoinositides are likely to be just “floating around”. It is possible that they form some micelle like structure, however one would imagine that this type of chemical structure would also be efficiently solubilised by detergents. More likely, phosphoinositides are sequestered by proteins that interact with and hide their hydrophobic tails but are able to present the inositol head group for further phosphorylation or phospholipase C mediated cleavage.

**PtdIns(4,5)P_2 signalling in the nucleus**

Within the nucleus PtdIns(4,5)P_2 is central to phosphoinositide signalling being a substrate for both phospholipase C (PLC EC 3.1.4.11) and phosphatidylinositol-3-kinase (PtdIns-3-kinase EC 2.7.1.153) and is itself a second messenger (van den
Bout and Divecha, 2009). We and others demonstrated that stimulation of Swiss 3T3 cells with IGF-1 led to a decrease in PtdIns(4,5)P2 and PtdIns4P levels with a consequent increase in nuclear Diacylglycerol (Divecha et al., 1991; Cocco et al., 1988). The simplest interpretation was that IGF-1 could stimulate the activity of a nuclear phospholipase C. Consequently it was shown that Phospholipase C b1 is present in nuclei (Martelli et al., 1992; Divecha et al., 1993) and that it can be phosphorylated at serine-982 by MAP kinase (EC 2.7.11.24) in response to IGF-1 stimulation (Xu et al., 2001). Interestingly, overexpression of a mutant that is unable to be phosphorylated by MAP kinase attenuates the increase in nuclear phospholipase C activity and attenuates IGF-1 induced proliferation. How does increased nuclear phospholipase C regulate cell proliferation? Interestingly, PLCb1 has been implicated in the regulation of theJun AP1 complex, although how this occurs is not clear (Ramazzotti et al., 2008). Phospholipase C mediated cleavage of PtdIns(4,5)P2 generates diacylglycerol (DAG) and inositol(1,4,5)trisphosphate (Ins(1,4,5)P3). DAG is a potent activator of Protein kinase C (Nishizuka, 1984), which translocates to the nucleus in response to IGF-1 stimulation (Divecha et al., 1991; Banfic et al., 1993; Martelli et al., 1991). There are many nuclear substrates of PKC, however, which if any regulate proliferation in response to IGF-1 stimulation is not clear. The other second messenger, Ins(1,4,5)P3 can regulate a number of pathways. Ins(1,4,5)P3 receptors that regulate calcium flux have been found on the inner nuclear envelope (Malviya et al., 1990; Humbert et al., 1996) and recent studies have suggested that nuclear Ins(1,4,5)P3 may specifically mediate increases in nuclear calcium (Rodrigues et al., 2007, 2008, 2009; Gomes et al., 2008). Increased nuclear calcium could potentially regulate an array of transcriptional regulators to modulate proliferation (Bading et al., 1997; Hardingham et al., 1997). Ins(1,4,5)P3 can also be further phosphorylated in the nucleus to generate a number of highly phosphorylated inositols. These are water soluble second messengers, which have been implicated in the control of chromatin remodelling, mRNA export and telomere function (Tsui and York, 2010). In a similar manner to phosphoinositides, inositol phosphates also regulate protein function by specifically interacting with protein domains. In this case they are unlikely to regulate localisation, but are more likely to modulate protein conformation which in turn regulates their activity and function. At the plasma membrane PtdIns(4,5)P2 can also act as a second messenger. It can interact with and regulate the function of ion channels, actin binding proteins, focal adhesion and endocytic complex components and pro-apoptotic proteins (van den Bout and Divecha, 2009). For PtdIns(4,5)P2 to function as a specific second messenger within the nucleus we expect that PtdIns(4,5)P2 levels would be controlled by nuclear specific factors and that there would be nuclear specific downstream targets. PtdIns(4,5)P2 can be synthesised by two different enzyme families that are highly related, Phosphatidylinositol-4-phosphate (PtdIns4P)-5-kinases (PIP5Ks EC 2.7.1.68) phosphorylate PtdIns4P (Loijens et al., 1996) on the 5-position while phosphatidylinositol-5-phosphate (PtdIns5P)-4-kinases (PIP4Ks
EC 2.7.1.149) phosphorylate PtdIns5P on the 4-position (van den Bout and Divecha, 2009). So which family is responsible for the synthesis of PtdIns(4,5)P$_2$ in the nucleus? Isoforms of both families are present in the nucleus (Ciruela et al., 2000; Boronenkov et al., 1998; Mellman et al., 2008), however, the mass level of PtdIns4P is at least 20 fold higher than the level of PtdIns5P. This suggests that PtdIns(4,5)P$_2$ is synthesised primarily through the PIP5K pathway. In order to further analyse this we incubated isolated nuclei with radiolabelled ATP for short time periods and then isolated the PtdIns(4,5)P$_2$ and determined on which position the label was incorporated. We found that the relative labelling ratio of the 5 to the 4 position was approximately 1.8 (Vann et al., 1997). There are two possible interpretations to these experimental data. The first is that approximately two times more PtdIns(4,5)P$_2$ is synthesised through the PIP5K than the PIP4K pathway (we cannot determine if the radiolabel was on the same molecule or on different molecules of PtdIns(4,5)P$_2$). The second is that the labelling of the 4-position occurs because of new synthesis of PtdIns4P from PtdIns phosphorylation, which is passed on to PIP5K for synthesis of PtdIns(4,5)P$_2$. In order to differentiate between these possibilities, we undertook a similar nuclear labelling experiment in the presence of inhibitors of the enzymes that can synthesise PtdIns4P, PI4K (EC 2.7.1.67). High concentrations of wortmannin inhibit the PI4KIII family of enzymes while adenosine is a specific inhibitor of the PI4KII family (Balla and Balla, 2006). Interestingly, both inhibitors blocked PtdIns4P synthesis to about 50% each in isolated intact nuclei and to approximately 90% when incubated in combination. When we determined the ratio of radiolabelling of the 5 to the 4 position of PtdIns(4,5)P$_2$ labelled in the presence of the inhibitors we found that treatment with wortmannin increased the ratio to 10:1 while adenosine had no effect. Neither wortmannin nor adenosine had any effect on the in vitro activity of the PIP4K enzymes. These simple and elegant in vitro studies suggest that in nuclei there are two families of enzymes that synthesise PtdIns4P, but that only the wortmannin sensitive enzymes provide PtdIns4P that is used by the PIP5K to generate PtdIns(4,5)P$_2$. Furthermore it would appear that at least 90% of nuclear PtdIns(4,5)P$_2$ is derived from the PIP5K pathway with the PIP4K pathway possibly providing a small minority of the nuclear PtdIns(4,5)P$_2$. These data suggest that PtdIns4P generated by the adenosine sensitive enzyme may be involved in direct signalling, while the PtdIns4P synthesised from the wortmannin sensitive enzyme may be required for the synthesis of PtdIns(4,5)P$_2$. The data also suggest that the role of the PIP4K may not be related to their ability to generate PtdIns(4,5)P$_2$ but that they may have a more specialised function in the nucleus (see later).

**Nuclear specific regulators of PtdIns(4,5)P$_2$ synthesis**

It is still unclear which isoforms of PIP5K are present in the nucleus. This is in part a consequence of the lack of suitable antibodies and because when overexpressed in cells, PIP5K generally localise to the plasma-membrane. However, PIP5K$_\alpha$ (Mellman et al., 2008; Boronenkov et al., 1998) and two splice variants of PIP5K$_\gamma$ have been shown...
to be present in the nucleus. How their localisation is regulated is not clear. However, two nuclear specific regulators of PIP5K have been defined. We initially demonstrated that the Retinoblastoma protein (pRB) interacts with all isoforms of PIP5K (Divecha et al., 2002). This was very exciting as pRB is a nuclear localised master regulator of differentiation, cell survival and progression through the cell cycle. More over the pRB pathway is deregulated in some manner in nearly all human tumours. pRB interacts with all isoforms of PIP5K and the interaction stimulates PIP5K activity. To demonstrate that PtdIns(4,5)P₂ synthesis in the nucleus is regulated by pRB, we first demonstrated that large T antigen, a viral oncoprotein able to inactivate and sequester pRB, was able to block the interaction between pRB and PIP5K. Using a temperature sensitive mutant of large T antigen stably expressed in MEL cells, we showed that when large T sequesters pRb there is a decrease in the mass of nuclear PtdIns(4,5)P₂. In fact we have shown that pRb acts as a scaffold protein for a number of different enzymes involved in phosphoinositide regulation including PIP4K and the specific zeta isoform of Diacylglycerol kinase (Los et al., 2006). pRB also interacts with the p55 regulatory subunit of PI-3-kinase (Xia et al., 2003). Fig. 2.

Another well characterised regulator of PIP5K has emerged from the Anderson laboratory. Using Yeast two hybrid analysis, Star-Pap was identified as an interactor with PIP5Kα (Mellman et al., 2008). Interaction regulates the localisation of PIP5Kα to nuclear speckles, where PtdIns(4,5)P₂, presumably synthesised by PIP5Kα, regulates the activity of Star-Pap. Star-Pap is a poly(A) polymerase that regulates the length

Figure 2. The retinoblastoma protein acts a scaffold for phosphoinositide metabolism. PIP5K, PIP4K and DAG kinase δ interact with the pRB protein. PLCb1 was not found to interact with pRB. In vitro we showed that addition of pRB activated PIP5K and DAGK activity, while inhibiting PIP4K activity. In vivo attenuating the interaction between PIP5K and pRB led to a decrease in nuclear but not cytoplasmic PtdIns(4,5)P₂. The dark blue shapes are potential targets that may be affected by pRB mediated regulation of phosphoinositides in the nucleus. (for interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
of the poly-A tail of a select set of mRNAs, some of which appear to be involved in regulating responses to oxidative stress. RNAi mediated suppression of PIP5K leads to a decrease in the levels of a subset of mRNA that are also regulated by Star-Pap. The data suggest that the Star-PAP complex acts as a hub for nuclear PtdIns(4,5)P₂ signalling to control the response to oxidative stress. Interestingly, pRB is also critical for responses to oxidative damage and thus may impinge on the Star-PAP pathway through regulation of PtdIns(4,5)P₂ synthesis. Whether Star-PAP is directly regulated by PtdIns(4,5)P₂ is not clear as star-PAP is also regulated by phosphorylation by Casein kinase 1, an enzyme that is also regulated by PtdIns(4,5)P₂ (Gonzales et al., 2008).

Thus far few nuclear specific PtdIns(4,5)P₂ interactors have been identified. Using a number of different strategies to enrich and purify PtdIns(4,5)P₂ interacting proteins from the nuclei of MEL cells, we have identified an enrichment of proteins that are part of the pre-mRNA and mRNA splicing complexes and in proteins involved in regulating DNA damage responses. Clearly further definition of PIP5K and PtdIns(4,5)P₂ interactors will be critical for understanding the complexity of the PtdIns(4,5)P₂ synthesis in the nucleus.

**PtdIns(5)P signalling in the nucleus**

The presence of the PIP4K family in the nucleus suggests that either they regulate a small minor pool of PtdIns(4,5)P₂ or that they can regulate the level of their substrate PtdIns5P. Using specific assays we showed that PtdIns5P is present in the nucleus and that its levels increase when PIP4K enzymes are suppressed using RNAi (Jones et al., 2006). We have also demonstrated that in Caenorhabditis elegans and in Drosophila, knockout of the single PIP4K enzyme leads to increased levels of PtdIns5P, without significant changes in the levels of PtdIns(4,5)P₂.

These data suggest that in vivo the role of PIP4K is to regulate PtdIns5P levels. The level of nuclear PtdIns5P is increased in response to oxidative stress and UV treatment and this occurs downstream of the activation of the stress activated p38 pathway (Jones et al., 2006). So what are the consequences of increased PtdIns5P in the nucleus? A seminal paper from Gozani et al. (Gozani et al., 2003) demonstrated that the PHD finger of the growth inhibitory protein 2 (ING2) was able to interact with PtdIns5P. This paper was the first to define a protein domain that could interact with PtdIns5P. What is really interesting about this is that PHD fingers are generally only found in nuclear proteins many of which are involved in regulating gene transcription through the modulation of chromatin structure. ING2 also regulates the level of acetylation of the tumour suppressor p53 and increases in PtdIns5P induced acetylation and activation of p53 in a stress dependent manner. P53 is a master regulator of cell proliferation and is highly mutated and inactivated in human tumours. These data therefore link stress activated modulation of nuclear PtdIns5P to the function of an important human tumour suppressor gene. To determine how common PHD interaction with phosphoinositides is, we have cloned over thirty of them and have assessed them for interaction with phosphoinositides. We find that of
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these approximately 10 interact strongly with phospholipids. Some PHD fingers also interact with trimethylated lysine 4 of histone H3 suggesting that they can translate the histone code into changes in chromatin structure and gene expression (Shi et al., 2006). However, of the thirty PHD fingers that we have cloned only four showed interaction with peptides containing trimethylated lysine 4 of histone H3. A subset of PHD fingers interact both with phosphoinositides and with modified histones, however it is not clear if phosphoinositide interaction can modulate or compete for histone interaction. We have also found that PtdIns5P can also regulate the levels of trimethyl-lysine 4-histone H3 at the nucleosome of some promoters and therefore modulate gene expression. The PHD finger of ATX1, a plant homologue of the mammalian trithorax proteins, does not interact with Trimethyl-lysine 4 of H3 but shows exquisite preference for interaction with PtdIns5P (Alvarez-Venegas et al., 2006) ATX1 contains a SET domain that can trimethylate lysine 4 of histone H3. Using expression arrays the WRKY70 gene was shown to be regulated by both ATX1 and by increased levels of PtdIns5P. The expression of WRKY70 and the level of trimethylated lysine 4 of histone H3 on nucleosomes around its promoter was used to study how changing PtdIns5P modulated ATX1 activity in vivo. We showed that drought stress induced an increase in total levels of cellular PtdIns5P, that was mediated by the plant homologue of myotubularin, a PtdIns(3,5)P2 3-phosphatase. The increase in PtdIns5P led to a decrease in the presence of the trimethyl mark on lysine 4 of histone H3 at the promoter of WRKY70. Using CHIP analysis we found that increased PtdIns5P also led to a decrease in the levels of ATX1 associated with promoters. In fact using immunofluorescence microscopy we showed that increased cellular PtdIns5P led to a change in the localisation of ATX1 from the nucleus to the cytoplasm which was dependent on the integrity of the PHD finger. Other PHD fingers also interact with a different subset of histone marks including acetylated histones (Zeng et al., 2010; Matsuyama et al., 2010) and again according to our own data some of these are also able to interact with phosphoinositides. The data suggest that nuclear PtdIns5P levels may have an important role in modulating where and to what extent PHD finger containing proteins are activated and how they then impinge on chromatin structure and gene expression.

So how are the levels of nuclear PtdIns5P regulated? As alluded to earlier, nuclear PtdIns5P levels are under the control of the stress activated p38 pathway (Jones et al., 2006). We found that a PIP4K activity that can phosphorylate and remove PtdIns5P, is present in the nucleus and is regulated by p38 in a stress dependent manner. There are three isoforms of PIP4K, α, β and γ and we showed that the α isoform was predominantly cytosolic, while the β isoform was cytosolic and nuclear (Ciruela et al., 2000). We showed that PIP4Kβ was directly phosphorylated by p38 at serine 326 and that phosphorylation led to a decrease in PIP4K activity associated with PIP4Kβ. To demonstrate that PIP4Kβ controls nuclear PtdIns5P levels, we showed that overexpression of PIP4Kβ decreased, while RNAi mediated suppression increased
nuclear PtdIns5P (Jones et al., 2006). Interestingly, detailed analysis of the difference in the activities of the three isoforms of PIP4K, α, β and γ, showed that PIP4Kβ has two thousand times less PIP4K activity compared to PIP4Kα (Wang et al., 2010; Bultsma et al., 2010). How then does PIP4Kβ, which has very little PIP4K activity, regulate nuclear PtdIns5P? To begin to understand this we immunoprecipitated PIP4Kβ from cells and identified associated proteins using mass spectrometry. Interestingly, PIP4Kβ associates with PIP4Kα. We then carried out a series of experiments to demonstrate that in vivo the majority of PIP4K activity in a PIP4Kβ immunoprecipitate was actually derived from its association with PIP4Kα (Bultsma et al., 2010). Our previous data demonstrated that PIP4Kα was actually a cytosolic enzyme while PIP4Kβ was a nuclear enzyme. So how does PIP4Kβ regulate nuclear PtdIns5P levels? We found that when co-overexpressed PIP4Kβ was able to target the activity of PIP4Kα to the nucleus (Bultsma et al., 2010). So, while things appear more complicated than at first view, it does appear that PIP4Kβ is important in regulating nuclear PtdIns5P levels. How phosphorylation by the p38 pathway regulates PIP4K activity associated with PIP4Kβ is not clear but it may regulate the association between PIP4Kα and PIP4Kβ.

While PIP4Ks are able to regulate PtdIns5P levels by phosphorylating it to PtdIns(4,5)P$_2$, what is really unclear is how PtdIns5P is synthesised. There are other enzymatic activities, present in the nucleus, which could synthesise PtdIns5P. The PIP5K family can synthesise PtdIns5P from PtdIns, albeit very inefficiently. PtdIns5P can also be generated by dephosphorylation of PtdIns(4,5)P$_2$ and a PtdIns (4,5)P$_2$-4-phosphatase (EC 3.1.3.78) has been characterised in mammalian cells that translocates to the nucleus upon stress induction (Ungewickell et al., 2005; Zou et al., 2007). Finally myotubularins can dephosphorylate PtdIns(3,5)P$_2$ to generate PtdIns5P (Coronas et al., 2008; Walker et al., 2001) although PtdIns(3,5)P$_2$ has not been demonstrated in the nucleus. Alternatively, and perhaps more interesting, there may be a novel enzymatic activity that synthetises nuclear PtdIns5P.

**Conclusions**

While we have a picture of phosphoinositide signalling in the nucleus there are many aspects to it that we just don’t understand. We have no clue as to how phosphoinositides enter the nucleus or to the identity of the putative proteins that may sequester phosphoinositides in the nucleus. How do phosphoinositides gain access to these proteins? Are there factors that can load phosphoinositides onto these proteins? Does this occur in the nucleus or outside of the nucleus? Which phosphoinositide is loaded onto the protein? What happens after phospholipase C mediated cleavage? How is the phosphoinositide resynthesised?

What is also very unclear is how do hot spots of nuclear phosphoinositides regulate the function of histone interacting proteins. One could imagine a number of different possibilities. The lipid hot spots may act as hubs where chromatin loops
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out and nucleosomes and phosphoinositides are brought together. Hubs of this type have been described where transcription occurs (Malyavantham et al., 2008a,b), however, whether phosphoinositides are also present remains to be determined. An alternative is that the hot spots function rather like a drive through. Changes in phosphoinositides induce the recruitment of a protein to the hotspot. Interaction with the phosphoinositide may induce modification of the protein such as phosphorylation or acetylation, which may change the location, interaction partners or the activity of the protein.

The complexity of nuclear inositide signalling suggests that it is likely to be involved in the regulation of most if not all nuclear processes. How phosphoinositides function within the nucleus requires that we understand how their levels are regulated and how the changes in nuclear phosphoinositides are transduced into output signals. This really means that we need to identify specific nuclear proteins that interact with and are regulated by phosphoinositides. These are not simple tasks but the fact that nuclear phosphoinositides control gene expression, mRNA stability and export and chromatin remodelling and that specific regulatory factors are required that are only utilised within the nuclear compartment, means that the rewards are likely to be great. As nuclear phosphoinositides impinge on the pRB, p53 pathway and on chromatin structure, we expect that unravelling the complexity of nuclear inositide signalling will offer up some new insights into the development of novel targets for cancer therapy.

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