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Review

Regulation of nuclear phospholipase C activity★

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A body of evidence, linking inositide-specific phospholipase C (PI-PLC) to the nucleus, is quite extensive. The main isoform in the nucleus is PI-PLCβ1, whose activity is up-regulated in response to insulin-like growth factor-1 (IGF-1) or insulin stimulation. Whilst at the plasma membrane this PI-PLC is activated and regulated by Gαq/α_{11} and G$\beta\gamma$ subunits, there is yet no evidence that qα/α_{11} is present within the nuclear compartment, neither GTP-γ-S nor AlF4 can stimulate PI-PLCβ1 activity in isolated nuclei. Here we review the evidence that upon occupancy of type 1 IGF receptor there is translocation to the nucleus of phosphorylated mitogen-activated protein kinase (MAPK) which phosphorylates nuclear PI-PLCβ1 and triggers its signalling, hinting at a separate pathway of regulation depending on the subcellular location of PI-PLCβ1. The difference in the regulation of the activity of PI-PLCβ1 mirrors the evidence that nuclear and cytoplasmatic inositides can differ markedly in their signalling capability. Indeed, we do know that agonists which affect nuclear inositol lipid cycle at the nucleus do not stimulate the one at the plasma membrane.

Even though polyphosphoinositides are quantitatively minor constituents of cell membranes, they are key players in signal transduction. It is well known that phosphoinositide signalling involves the generation of lipid second messengers, phosphati-

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Abbreviations: DGK, diacylglycerol kinase; IGF-1, insulin-like growth factor-1; MAPK, mitogen-activated protein kinase; PI-PLC, phosphoinositide-specific phospholipase C; PMA, phorbol 12-myristate 13-acetate; PtdIns, phosphatidylinositol.

dylinositol 4-phosphate (PtdIns(4)P) and phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2), in response to stimuli in a receptor-mediated manner at the plasma membrane. More recently it appeared clear that a nuclear polyphosphoinositol metabolism exists independently of that at the plasma membrane (Martelli *et al.*, 1999). In fact, it has been shown by several laboratories that nuclei contain many enzymes involved in the phosphatidylinositol (PtdIns) cycle, including kinases required for the synthesis of PtdIns(4,5)P_2, specific phospholipase C (PI-PLC), and diacylglycerol kinase (DGK) (D'Santos *et al.*, 1998; Cocco *et al.*, 2001; Irvine, 2003). Specific changes in the nuclear PtdIns metabolism have been implicated in cell growth, differentiation, and tumour progression (Divecha *et al.*, 2000; Tamiya-Koizumi, 2002; Martelli *et al.*, 2000).

In this review, we shall highlight the regulatory mechanisms of nuclear PI-PLCβ1 upon stimulation of Type 1 IGF receptor in quiescent cells.

EVIDENCE FOR THE PRESENCE AND SIGNALLING ACTIVITY OF PI-PLCβ1 IN THE NUCLEUS

We know now that PI-PLCs can be found in the nucleus and that the resident isoform is PI-PLCβ1 (Cocco *et al.*, 2001). The evidence of both the presence and enzymatic activity of a nuclear PI-PLC in quiescent Swiss 3T3 mouse fibroblasts, mitogenically stimulated with insulin-like growth factor-1 (IGF-1), stems from data showing that in membrane-stripped nuclei IGF-1 produced a decrease in PtdIns(4)P and PtdIns(4,5)P_2 mass and a concomitant increase in DAG levels, within 2 min stimulation (Divecha *et al.*, 1991). Thus, activation of a nuclear PI-PLC seemed likely. No changes in PtdIns(4)P, PtdIns(4,5)P_2, and diacylglycerol (DAG) amount were detected in whole cell homogenates or in nuclei in which the envelope was still present. Bombesin, another powerful mitogen for these cells, stimulated inositide metabolism at the plasma membrane level (as demonstrated by changes in the DAG mass measured in whole cell homogenates), but not in the nucleus. Concomitantly, our group demonstrated the presence, in nuclei of Swiss 3T3 mouse fibroblasts, of PI-PLCβ1 whose activity was up-regulated in response to IGF-1 stimulation (Martelli *et al.*, 1992).

Nuclear PI-PLCβ1 plays an important role as a mediator of the mitogenic stimulus exerted by IGF-1 on Swiss 3T3 mouse fibroblasts, since inhibition of PI-PLCβ1 expression by antisense RNA renders these cells far less responsive to IGF-1, but not to platelet-derived growth factor (Manzoli *et al.*, 1997). As a result of the increase in intranuclear DAG mass, PKC-α migrates to the nucleus (Neri *et al.*, 1994). An important issue is to link PKC-α to normal cell proliferation. Recent findings have pointed out that, in NIH 3T3 mouse fibroblasts treated with powerful tumor promoter phorbol 12-myristate 13-acetate (PMA), PKC-α and PKC-ε activate cyclin D1 and cyclin E promoters and thus markedly elevate the levels of both cyclin D1 and E. This results in higher rate of cell proliferation. Up-regulation of cyclin D1 expression is mainly mediated through the AP-1 transcription factor enhancer element present in the cyclin D1 promoter (Soh *et al.*, 2003). Interestingly stimulation of type 1 IGF receptor, upon binding of IGF-1 or insulin, affects nuclear PI-PLCβ1 also in differentiation. Indeed, differentiation of C2C12 myoblasts in response to insulin stimulation is characterized by a marked increase in nuclear PI-PLCβ1 (Faenza *et al.*, 2003). The timing of PI-PLCβ1 synthesis and its accumulation in the nucleus precedes that of the late muscle marker troponin T by 24 h and the expression of a transfected PI-PLCβ1 mutant lacking the nuclear localisation signal acts as a dominant negative for nuclear translocation of PI-PLCβ1 and suppresses the differentiation of C2C12 myoblasts.

It is known that myogenic factors regulate not only tissue-specific gene expression but also the exit from the cell cycle. At the onset of differentiation, MyoD activates cyclin D3 which then sequesters unphosphorylated retinoblastoma protein leading to irreversible exit of differentiating myoblasts from the cell cycle (Cenciarelli *et al.*, 1999). This fits with our previous observations showing that a downstream target of nuclear PI-PLCβ1 signalling is the cyclin D3/cdk4 complex (Faenza *et al.,* 2000).

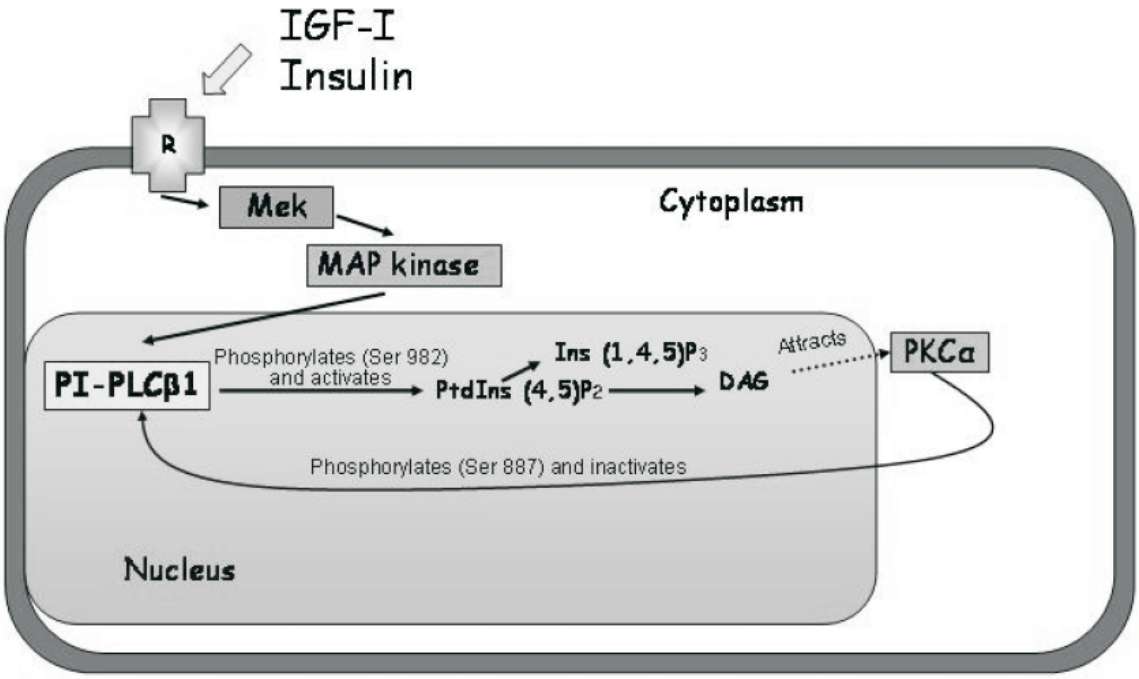

Figure 1. Schematic diagram showing the main features of nuclear PI-PLCβ1 regulation.

Upon activation of Type-1 IGF receptor in quiescent mouse fibroblasts, miotogen kinase (Mek) phosphorylates mitogen-activated protein kinase (MAP kinase), which in turn phosphorylates PI-PLCβ1 on serine-982 (Ser-982). This event activates PI-PLCβ1 which hydrolyses PtdIns(4,5)P_2 and DAG are produced within 5–15 min of IFG-1 treatment. As a result of the increase in intranuclear DAG mass, PKC-α migrates to the nucleus and phosphorylates PI-PLCβ1 on serine-887 (Ser-887). This second phosphorylation, which occurs after 30 min of IGF-1 stimulation, deactivates PI-PLCβ1.

REGULATION OF NUCLEAR PI-PLCβ1 ACTIVITY

Until few years ago, the regulation of nuclear PI-PLCβ1 activity was still obscure. The conventional view of PI-PLCβ1 activation is based on the data obtained by studying its action at the plasma membrane. It has been suggested that both Gαq/α_{11} and G$\beta\gamma$ subunits can activate PI-PLCβ1. The region of PI-PLCβ1 that interacts with Gαq differs from that which interacts with G$\beta\gamma$, the former binding to the extensive C-terminal tail characteristic of the PI-PLCβ isoforms, while the latter has highest affinity for the N-terminal PH domain (Rhee, 2001). There is yet no evidence that αq/α_{11} is present within the nuclear compartment. Consistently, GTP-γ-S did not stimulated PI-PLCβ1 *in vitro* activity in nuclei of murine erythroleukaemia (MEL) cells (Martelli *et al.*, 1996). A clue to a possible novel mechanism for activation of nuclear PI-PLCβ1 has come from the observation that it is hyperphosphorylated in Swiss 3T3 mouse fibroblast nuclei in response to IGF-1 and that this is abolished by preventing the

translocation of p42/44 mitogen-activated protein kinase (MAPK) to the nucleus (Martelli *et al.*, 1999). Evidence was obtained with both insulin-treated NIH 3T3 mouse fibroblasts (Martelli *et al.*, 2000) and IL-2 treated human primary natural killer cells (Vitale *et al.*, 2001) where activation of nuclear PI-PLCβ1 was blocked by PD098059, an inhibitor selective for the MAPK pathway. Definitive proof of a direct involvement of MAPK has come from the demonstration that, following IGF-1 stimulation of quiescent Swiss 3T3 mouse fibroblasts, activated p42/44 MAPK translocates to the nucleus where it phosphorylates Ser-982 in the C-terminal domain of PI-PLCβ1 (Xu *et al.*, 2001). This phosphorylation was inhibited by PD098059 and could be mimicked by recombinant PI-PLCβ1 protein and activated MAPK *in vitro*. This may represent an activation mechanism which is distinct from that at the plasma membrane and peculiar to the actions of the nuclear phosphoinositide cycle. In this regard, it may be significant that within the PI-PLCβ family, the β1 isoform is the only one which possesses a MAPK phosphorylation site in its C-terminal tail.

These are data suggesting that PI-PLCβ1 is deactivated by PKC-α and that this is a critical step in attenuating the phospholipase activity that drives the nuclear inositol lipid cycle (Cocco *et al.*, 2002). It would be interesting to know the effects of this point mutation of PKC-α on IGF-I-evoked mitogenesis.

All in all, the data discussed in this review strengthen the contention of a direct link between occupancy of type 1 IGF receptor, translocation to the nucleus of activated MAPK and stimulation of the nuclear PI-PLCβ1 signalling, which targets specific transcription factors involved in G1 progression and differentiation as well.

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