MINIREVIEW

Nuclear Lipid Signaling: Novel Role of Eicosanoids

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Nuclear lipid signaling is an established, widespread mechanism that operates in multiple cellular processes including proliferative and differentiative responses to a variety of stimuli. In this literature review with key references highlighted, we put forward the hypothesis that differential flow through various intracrine mechanisms can dictate resultant cellular actions such as mitosis, differentiation, or apoptosis. [E.B.M. 2001, Vol 226:1–4]

Key words: signalling; eicosanoids; second messengers; nucleus; intracrine

It is now more than 10 years since the first publication on nuclear signaling (1). Initially the observations were limited to the actions of insulin-like growth factor-1 (IGF-1) that generates, in addition to conventional cytoplasmic tyrosine kinase signaling cascades, a distinct and autonomous nuclear signaling pathway shown to be essential for the initiation of cell division (2).

The basis of the nuclear signal by IGF-1 is the local production of the second messenger diacylglycerol (DAG), a potent activator of protein kinase C, by hydrolysis of a nuclear pool of polyphosphoinositides (3); that is, lipids that hitherto had been considered exclusive to plasma membrane signaling mechanisms.

These initial observations have now been extended to include proliferative and differentiative responses to a variety of stimuli in diverse systems, and it is now clear that nuclear lipid signaling is a firmly established and widespread mechanism that operates in multiple cellular processes (4–7).

Several plausible reasons explain why intracellular signaling might be compartmentalized this way. Depending on cell context, a pleiotropic hormone like IGF-1 can have profound effects on both protein and energy homeostasis as well as cell division and differentiation; therefore, it is important to distinguish clearly metabolic from mitogenic signals. In contrast, this ambivalence does not exist for steroid hormones whose effects arise from direct interactions at the gene level. Another cogent argument is that a multistepped signaling pathway provides numerous foci for further modification by interactive cross-talk with other signals; however, cells are rarely exposed *in vivo* to a single stimulus. Cellular responses reflect the end point of multiple stimuli as they pass through the mesh of the intracellular signaling network. Nuclear signaling might represent an exclusive output from this process reserved for important decisions on cell proliferation, determination, and fate. The aim of this article is to provide a brief outline of the current status of nuclear signaling and the role of nuclear glycerophospholipids as a source of second messengers. Particular consideration is given to the metabolites of specific fatty acids known collectively as eicosanoids as potential new players in nuclear signaling mechanisms.

Nuclear Lipids

Central to this discussion is the existence of a discrete pool of nuclear glycerophospholipids that is metabolically distinct from analogous lipids in the plasma membrane. Biochemical evidence suggests that most of the lipids are present in the nuclear envelope, but phosphatidylinositol 4,5-bisphosphate [PdtIns $(4,5)P_2$] differs in that about half of its mass appears to be truly intranuclear (8). PtdIns $(4,5)P_2$ has also been located by immunochemical means to the internal nuclear matrix using a specific monoclonal antibody (9). Furthermore, immunoelectron microscopy suggests that the nuclear matrix also appears to harbour the phosphoinositol-specific phospholipase $C\beta_1$ ($PLC\beta_1$) whose activation is thought to be responsible for the production of DAG from intranuclear inositol phospholipids (10). However, nuclear DAG can be derived from glycerophospholipids other than those containing inositol. The hydrolysis of phosphatidylcholine (PdtCho) either by a choline-specific PLC or by a combination of phospholipase D

Funding was provided by The Health Research Council, Royal Society Marsden Fund, Lottery Health and Auckland Medical Research Foundation.

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0037-9727/01/2261-0000$15.00/0

(PLD) and phosphatidic acid (PA) phosphohydrolase can generate DAG, and indeed this appears to be the preferred route for nuclear DAG production and resultant PKC nuclear translocation in IIC9 fibroblasts in response to α-thrombin (11). However, in these studies nuclei were isolated with their envelopes intact. Since nuclear envelopes are enriched in PtdCho and also contain the bulk of nuclear DAG (8), this mechanism differs from PtdIns(4,5)P_2/PLC signaling in both lipid substrate and nuclear localization. Another important difference is the composition of the DAG products. The DAG derived from nuclear inositol lipids is mainly unsaturated, with over 70% of molecules containing two, four, and five double bonds whereas that derived from PtdCho is mainly mono- and disaturated (12). Analysis of these pools during cell cycle progression shows that they are independently regulated and presumably perform different regulatory roles. There is circumstantial evidence for this in that whereas most DAG species activate PKC *in vitro,* polyunsaturated DAGs are the most potent (13); the extent to which this relates to the *in vivo* situation is by no means clear. Indeed evidence that DAG is formed directly from PtdCho by a PC-specific PLC activity in mammalian cells is presently rather scant. A stronger case can be argued that the true signaling molecule is PLD-derived PA and that mono- and disaturated DAG are its inactive metabolites (14). Thus the predominant primary products of agonist-stimulated PLC and PLD activities are polyunsaturated DAGs and saturated/monosaturated PAs. These lipid messengers are removed rapidly from within the cell by the actions of DAG kinase and PA phosphohydrolase, respectively, and therefore the resulting polysaturated PAs and saturated/monosaturated DAGs are secondary metabolites rather than primary message. The ability of these pathways to act independently could be a function of compartmentalization of different DAG pools within the nucleus and/or specificity of DAG metabolism dictated by its fatty acid composition.

Arachidonic Acid

The metabolites of arachidonic acid, collectively known as eicosanoids, are an important class of second messengers with multiple biological actions. Only very small amounts of free arachidonic acid are found in cells, most of it being esterified to glycerophospholipids. Two principal methods are known to release arachidonate from phospholipids: direct hydrolysis by PLA2 or indirect generation of DAG through phospholipase action followed by mono- and diacylglycerol lipase action.

Early experiments implicated the nucleus as an important focus of arachidonate metabolism. For example, the nuclear membrane was found to have the highest specific activity following exposure of cells to labeled arachidonate (15); in mouse fibrosarcoma cells treated with bradykinin, arachidonate is preferentially released from phospholipids most recently incorporated into the nuclear membrane (16). Furthermore, many of the enzymes involved in the metabolism of arachidonic acid have been found within, or closely associated with, the nucleus. Nuclear PLA2 has been identified in rat liver (17) and rat hepatoma cells (18). Other evidence shows that PLA2 can move from the cytoplasm to the nucleus in a variety of cell types in response to a number of different stimuli (19–21). The nuclear translocation and concomitant activation of PLA2 has been shown to be mitogen-activated protein (MAP) kinase–dependent (22, 23). Further metabolism of arachidonate within the nucleus is suggested by the presence of some of the key enzymes involved. Thus the inducible form of prostaglandin H synthase (PGHS-2) is found to localize extensively with the nuclear envelope in Swiss 3T3 cells (24) and WISH amnion cells (25), and PGHS-2 and 5- and 12-lipoxygenases have also been found in the nuclei of luteal cells (26). Nuclear eicosanoid production has been reported following treatment of HL60 cells with differentiating agents retinoic acid and vitamin D_3. Interestingly, undifferentiated cell nuclei do not metabolize arachidonate to a significant extent; however, on differentiation, a variety of nuclear eicosanoids are synthesized that differ depending on the agent used (27).

Numerous researchers have observed a preference for substrates containing arachidonic acid when cPLA2 is incubated with synthetic phospholipids containing various fatty acids at the *sn*-2 position of the glycerol backbone (28). Experiments using natural and synthetic membranes demonstrate that cPLA2 prefers polyunsaturated fatty acids, especially those with three *cis* double bonds between carbons 5 and 6, 8 and 9, and 11 and 12. Relative to arachidonate, other polyunsaturated fatty acids are present in low abundance in membranes; therefore, arachidonyl phospholipids are the major substrate for PLA2 in biological systems.

In terms of the nuclear glycerophospholipids discussed above, PtdIns4P, PtdIns(4,5)P_2, and their corresponding PA metabolites are potential substrates for nuclear PLA2 activity (1-stearoyl-2-arachidonylglycerol is not a PLA2 substrate). Definitive demonstration of linked arachidonate production and eicosanoid synthesis within the nucleus is still awaited; nonetheless, it is interesting to speculate on the potential of such a signaling pathway.

Prostanoids as Second Messengers

The mechanisms of action of prostanoids [prostaglandins (PGs) and thromboxanes] are becoming increasingly more complex. Original thinking was that their entry into the cell was, like steroids, direct without the involvement of cell surface receptors. However, the membrane receptors for PGE_2, $PGF_{2\alpha}$, thromboxane A_2, and prostacyclin have now been cloned, and their intracellular signaling mechanisms identified. The cyclopentenone PGs, PGA_2 and J_2, do not follow this pattern. It is now known that 15-deoxy-$\Delta^{12,14}$-PGJ_2 is a ligand for the orphan receptor peroxisome proliferator-activated receptor gamma (PPARγ) (29). PPAR α, β, and γ isoforms are now recognized as members of the nuclear receptor superfamily of transcription factors that

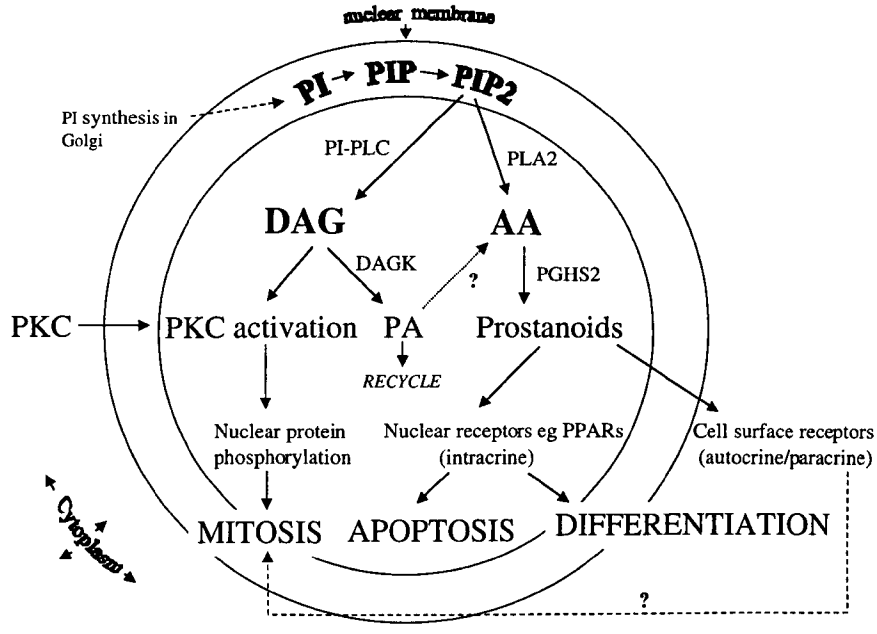

Figure 1. Schematic diagram of a proposed nuclear signaling mechanism.

includes the steroid receptors and therefore are capable of targeting the nucleus directly (30, 31).

Although natural ligands for PPARs have not been identified conclusively, the α isoform is activated by a variety of peroxisome proliferating agents and leukotriene B4 as well as by long-chain fatty acids (32–34). These agonists do not activate PPARγ (35); however, the arachidonic acid metabolite 15-deoxy-Δ^{12-14}-prostaglandin J_2(15d-PGJ_2) has been shown to be a natural ligand capable of inducing PPARγ-dependent adipogenesis (36). A key transcription factor in adipose development is ADD1/SREBP1 (37) that not only increases transcriptional activity of PPARγ but also stimulates the cell to produce endogenous ligand for PPARγ (38). To date the identification of this endogenous ligand has proved elusive; however, it is already clear that it is *not* 15d-PGJ_2 that fulfills this role *in vivo* (38). One possible source of ligand is from derivatives of exogenous fatty acids that *in vivo* could be dietary in origin. Another possibility is that the transition from cell proliferation to differentiation is accompanied by a switch from DAG production to arachidonate (and subsequently its metabolites) as the major signaling lipids of the nuclear PI cycle (Fig. 1). The effect of this switch would be to establish an intracrine signaling mechanism in which both receptor and agonist are synthesized and active within the confines of the single cell. We speculate that differential flow through this or similar intracrine mechanisms can dictate the resultant cellular action (e.g., mitosis, differentiation, apoptosis) (Fig. 1). Evidence from our group shows that the suppression of nuclear DAG synthesis is a feature common to erythroid differentiation (39, 40) as well as adipogenesis and myogenesis (unpublished data); however, no hard evidence exists as yet for the proposed alternative pathways of nuclear lipid metabolism.

Another example of where PPARγ appears to play a vital role in cell programming is in the initiation of apoptosis in gestational membranes at term and also at preterm as a result of complications, including intrauterine infection. The PPARδ is indeed expressed in human gestational tissues (41). It is currently believed that gestational membranes undergo terminal remodelling in preparation for parturition and that this involves increased apoptosis in amnion and trophoblast tissues toward term, a process that can occur prematurely in the presence of infection and indeed may be triggered by the same signals. We have reported the induction of apoptosis in JEG3 choriocarcinoma cells (42) and WISH amnion cells (43) in response to 15d-PGJ_2. Although both cell lines express PPARγ, it has yet to be established conclusively that apoptosis is mediated through this receptor. Indeed, in a recent report using microglial cells (44), the effects of 15d-PGJ_2 appeared to be PPARγ-independent, possibly mediated by the pro-inflammatory transcription factor NF-κB. Irrespective of the exact mechanism of 15d-PGJ_2 action, its production from PGD_2 raises questions about the source of the parent arachidonate and the programmatic steps that effect its appearance. One intriguing possibility is that this process, like that of adipogenesis, involves a switch in intracellular lipid signaling and that the nuclear PI pathway may be a key player in the process.

We are grateful to all of our colleagues who participated in the studies cited.

1. Cocco L, Martelli AM, Gilmour RS, Letcher AJ, Manzoli FA, Irvine RF. Changes in nuclear inositol phospholipids induced in intact cells by insulin-like growth factor I. Biochem Biophys Res Commun **159**:720–725, 1989.
2. Manzoli L, Billi AM, Rubbini S, Bavelloni A, Faenza R, Gilmour RS, Rhee SG, Cocco L. Essential role for nuclear phospholipase $C\beta_1$ in insulin-like growth factor-I induced mitogenesis. Cancer Res **57**:2137–2139, 1997.

3. Martelli AM, Gilmour RS, Neri LM, Manzoli L, Corps AN, Cocco L. Mitogen stimulated events in nuclei of Swiss 3T3 cells: Evidence for a direct link between changes of inositol lipids, protein kinase C requirement, and the onset of DNA synthesis. FEBS Lett **283:**243–246, 1991.
4. Cocco L, Martelli AM, Gilmour RS. Inositol lipid cycle in the nucleus. Cell Signal **6:**481–485, 1994.
5. Divecha N, Irvine RF. Phospholipid signaling. Cell **80:**269–278, 1995.
6. D'Santos CS, Clark JH, Divecha N. Phospholipid signaling in the nucleus. Biochim Biophys Acta **1436:**201–232, 1998.
7. Neri LM, Capitani S, Borgatti P, Martelli AM. Lipid signaling and cell responses at the nuclear level. Histol Histopathol **14:**321–335, 1998.
8. Vann LR, Wooding FB, Irvine RF, Divecha N. Metabolism and possible compartmentalization of inositol lipids in isolated rat liver nuclei. Biochem J **327:**569–576, 1997.
9. Mazzotti G, Zini N, Rizzi E, Rizzoli R, Galanzi A, Ognibene A, Santi S, Matteucci A, Martelli AM, Maraldi NM. Immunochemical detection of polyphosphotidyl inositol 4,5-bisphosphate localization sites in the nucleus. J Histochem Cytochem **43:**181–191, 1995.
10. Zini Martelli AM, Cocco L, Manzoli FA, Maraldi NM. Phosphoinositidase C isoforms are specifically localized in the nuclear matrix and cytoskeleton of Swiss 3T3 cells. Exp Cell Res **208:**257–269, 1993.
11. Jarpe MB, Leach KL, Raben DM. Alpha-thrombin-induced nuclear sn-1,2-diacylglycerols are derived from phosphatidyl choline hydrolysis in cultured fibroblasts. Biochemistry **33:**526–534, 1994.
12. D'Santos CS, Clarke JH, Irvine RF, Divecha D. Nuclei contain two differentially regulated pools of diacylglycerol. Curr Biol **9:**437–440, 1999.
13. Marignani PA, Epand RM, Sebaldt RJ. Alkyl chain dependence of diacylglycerol activation of protein kinase C *in vitro*. Biochem Biophys Res Commun **225:**469–473, 1996.
14. Wakelam MJO. Diacylglycerol: When is it an intracellular messenger? Biochim Biophys Acta **1436:**117–126, 1998.
15. Neufeld EJ, Majerus PW, Kreuger CM, Saffitz JE. Uptake and subcellular distribution of [^{3}H]arachidonic acid in murine fibrosarcoma cells measured by electron microscope autoradiography. J Cell Biol **101:**573–581, 1985.
16. Capriotti AM, Furth EE, Arrasmith ME, Laposata M. Arachidonate released on agonist stimulation preferentially originated from arachidonate most recently incorporated into nuclear membrane phospholipids. J Biol Chem **263:**10029–10034, 1988.
17. Neitcheva T, Peeva D. Phospholipid composition, phospholipase A2 and sphingomyelinase activities in rat liver nuclear membrane and matrix. Int J Biochem Cell Biol **27:**995–1001, 1995.
18. Tamiya-Koizumi K, Umekawa H, Yoshida S, Ishihara H, Kojima K. A novel phospholipase A2 associated with nuclear matrix: Stimulation of the activity and modulation of the Ca^{2+} dependency by polyphosphoinositides. Biochem Biophys Acta **3:**182–188, 1989.
19. Sierra-Honigmann MR, Bradley JR, Pober JS. "Cytosolic" phospholipase A2 is in the nucleus of subconfluent endothelial cells but confined to the cytoplasm of confluent endothelial cells and redistributes to the nuclear envelope and cell junctions upon histamine stimulation. Lab Invest **74:**684–695, 1996.
20. Fayard JM, Tessier C, Pageaux JF, Lagarde M, Laugier C. Nuclear location of PLA2–1 in proliferative cells. J Cell Sci **111:**985–994, 1998.
21. Freeman EJ, Ruehr ML, Dorman RV. ANG II-induced translocation of cytosolic PLA2 to the nucleus in vascular smooth muscle cells. Am J Physiol **274:**282–288, 1998.
22. Lin LL, Wartman M, Lin AY, Knopf JL, Seth A, Davis RJ. cPLA2 is phosphorylated and activated by MAP kinase. Cell **72:**269–278, 1993.
23. Kan H, Ruan Y, Malik KU. Involvement of mitogen-activated protein kinase and translocation of cytosolic phospholipase A2 to the nuclear envelope in acetylcholine-induced prostacyclin synthesis in rabbit coronary endothelial cells. Mol Pharmacol **50:**1139–1147, 1996.
24. Morita I, Schindler M, Regier MK, Otto JC, Hori T, DeWitt DL, Smith WL. Different intracellular locations for prostaglandin endoperoxidase H synthase-1 and -2. J Biol Chem **270:**861–870, 1995.
25. Marvin KW, Eykholt RL, Mitchell MD. Subcellular localization of prostaglandin H synthase-2 in a human amnion cell line: Implications for nuclear localized prostaglandin signaling pathways. Prostaglandins Leukot Essent Fatty Acids **62:**7–11, 2000.
26. Mitsuoka S, Otsura A, Nakao K, Tsutsumi T, Tsuruta S, Hamasaki K, Shima M, Nakata K, Tamaoki T, Nagataki S. Inhibitory effect of prostaglandin δ12-PGJ2 on cell proliferation and α-fetoprotein expression in HuH-7 human hepatoma cells. Prostaglandins **43:**189–197, 1992.
27. Matsumota K, Morita I, Murota S. Arachidonic acid metabolism by nuclei of a retinoic acid– or vitamin D3–differentiated human leukemia cell line HL-60. Prostaglandins Leukot Essent Fatty Acids **51:**51–55, 1994.
28. Clark JD, Schievella AR, Nalefski EA, Lin LL. Cytosolic phospholipase A_2. J Lipid Mediat Cell Signal **12:**83–117, 1995.
29. Forman BM, Tontonoz P, Chen J, Brun RP, Evans RM. 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 is a ligand for the adipocyte differentiation factor PPAR. Cell **83:**803–812, 1995.
30. Vamecq J, Latruffe N. Medical significance of peroxisome proliferator-activated receptors. Lancet **354:**141–148, 1999.
31. Auwerx J. PPARγ, the ultimate thrifty gene. Diabetologia **42:**1033–1049, 1999.
32. Gottlicher M, Windmark E, Li Q, Gustafsson JA. Fatty acids activate a chimera of the clofibric acid–activated receptor and the glucocorticoid receptor. Proc Natl Acad Sci U S A **89:**4653–4657, 1992.
33. Gulick T, Cresci S, Caira T, Moore DD, Kelly DP. The peroxisome proliferator-activated receptor regulates mitochondrial fatty acid oxidative enzyme expression. Proc Natl Acad Sci U S A **91:**11012–11016, 1994.
34. Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature **347:**645–650, 1990.
35. Kliewer SA, Foreman BM, Blumberg B, Ong ES, Borgmeyer U, Mangelsdorf DJ, Umesono K, Evans RM. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. Proc Natl Acad Sci U S A **91:**7355–7359, 1994.
36. Foreman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM. 15-deoxy-Δ^{1214}-prostaglandin J_2 is a ligand for the adipocyte determination factor PPARγ. Cell **83:**803–812, 1995.
37. Tontonoz P, Kim JB, Graves RA, Spiegelman BM. A novel helix-loop-helix transcription factor associated with adipocyte determination and differentiation. Mol Cell Biol **13:**4753–4759, 1993.
38. Kim JB, Wright HM, Wright M, Spiegelman BM. ADD1/SREBP1 activates PPARγ through the production of endogenous ligand. Proc Natl Acad Sci U S A **95:**4333–4337, 1998.
39. Manzoli L, Billi AM, Gilmour RS, Martelli AM, Matteucci A, Rubbini S, Weber G, Cocco L. Phosphoinositide signaling in the nuclei of Friend cells: Tiazofurin downregulates phospholipase $C\beta_1$. Cancer Res **55:**2978–2980, 1995.
40. Matteucci A, Faenza I, Gilmour RS, Manzoli L, Billi AM, Peruzzi D, Bavelloni A, Rhee SG, Cocco L. Nuclear but not cytoplasmic phospholipase Cβ inhibits differentiation of erythroleukemia cells. Cancer Res **58:**5057–5060, 1998.
41. Marvin KW, Eykholt RL, Keelan JA, Sato TA, Mitchell MD. The 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 receptor, peroxisome proliferator-activated receptor-γ (PPAR-γ) is expressed in human gestational tissues and is functinally active in JEG3 choriocarcinoma cells. Placenta **21:**436–440, 2000.
42. Keelan JA, Sato TA, Marvin KW, Lander J, Gilmour RS, Mitchell MD. 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, a ligand for peroxisome proliferator-activated receptor-γ, induces apoptosis in JEG3 choriocarcinoma cells. Biochem Biophys Res Commun **262:**579–585, 1999.
43. Nijmeijer BE, Soh EBA, Helliwell RJA, Mitchell MD, Gilmour RS, Keelan JA. PPAR-γ ligands induce apoptosis in amnion-derived WISH cells. J Soc Gynecol Invest **7:**139A, 2000.
44. Petrova TV, Akama KT, Van Eldik LJ. Cyclopentenone prostaglandins suppress activation of microglia: Downregulation of inducible nitric oxide synthase by 15-deoxy-$\Delta^{12,14}$ -prostaglandin J_2. Proc Natl Acad Sci U S A **96:**4668–4673, 1999.