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Topical Review

Nuclear Lipid Metabolism in NEST: Nuclear Envelope Signal Transduction

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Introduction

Since the early 1980's, there has been an explosion of research in an area often described as "signal transduction." Loosely defined, signal transduction refers to *the communication of a signal initiated by an extracellular agonist to the cell interior.* Clearly, such a process is central to the growth, development and homeostasis of multicellular organisms. Indeed, many extracellular agonists induce the stimulation of cell growth, differentiation, or the expression of specific genes required for selected responses. As a result, one of the primary intracellular targets of this communication is the cell nucleus. Signal transduction pathways must, therefore, include mechanisms for the initiation of signals at the plasma membrane, a mechanism by which these signals traverse the cytoplasm and influence, finally, a nuclear response.

Mechanisms by which cell surface-initiated signals impinge upon the nucleus have been the subject of numerous reviews ([26, 43, 47] and references therein). Most of the research has focused either on mechanisms involving cytoplasmic receptors, such as steroid receptors, or the generation of soluble second messengers that ultimately affect nuclear responses by modification of nuclear proteins and/or enzymes [26, 43, 47]. The mechanism by which tyrosine kinases mediate the transduction of signals from the plasma membrane, often via activation of serine/threonine kinases, has been a major emphasis of much of this research [14, 47].

While the above studies have provided valuable insights into the components likely to be involved in signal transduction pathways, little attention has been given to nuclear lipid metabolism as a participant in signaling cascades. Just as the plasma membrane serves as the communication link between the extracellular environment and the cytoplasm, the nuclear envelope is the barrier through which intracellular signals communicate with the nucleoplasm. In general, the nuclear envelope is composed of two membrane bilayers which meet at a complex of pore-forming proteins. In addition to the hypothesis that the nuclear pore may be involved in modulating nuclear responses (*see* [47]), there is now compelling evidence that agonist stimulation results in the induction of specific lipid metabolism in the nuclear membranes. Indeed, there is increasing evidence to support the provocative hypothesis that this lipid metabolism is an important component of what we define as NEST: *N*uclear *E*nvelope *S*ignal *T*ransduction. This metabolism, and some of its potential consequences, is the subject of this review.

Nuclear Lipid Metabolism: The Biochemistry

Metabolism of Nuclear Phosphoglycerides

Generation of Nuclear Diglycerides

It is now well established that induced increases in cellular diglycerides are an important component of numer-

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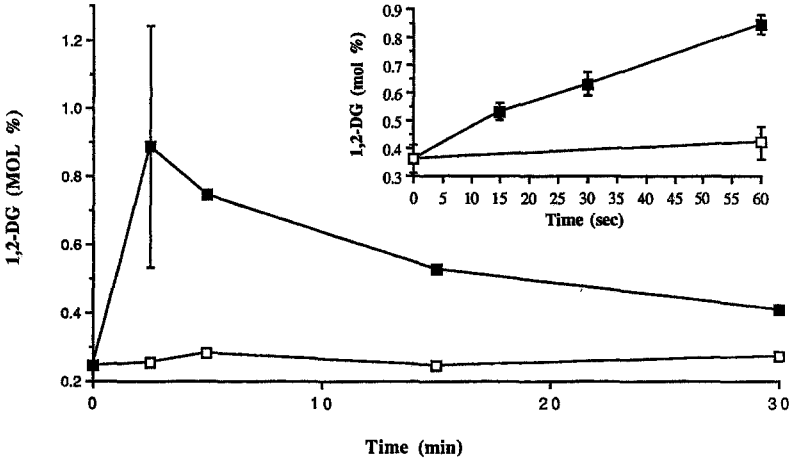

Fig. 1. Time course of diacylglycerol production in IIC9 nuclei. IIC9 cells were treated with (■) or without (□) 500 ng/ml α-thrombin (≈4,000 NIH U/mg) and at the indicated times, nuclei were prepared and nuclear diacylglycerol mass was quantified as previously described [33]. The results presented represent two independent experiments each performed in duplicate and are representative of at least six (main graph) or two (inset) independent experiments. Error bars are present on all data points and indicate the range of values. (Reprinted with permission from the *Journal of Biological Chemistry.*)

ous signal transduction pathways. The source of this diglyceride was initially thought to be derived exclusively from the hydrolysis of phosphoinositides resulting in the production of inositol phosphates, IP_3 in particular, and diacylglycerol leading to the elevation of intracellular calcium and activation of protein kinase C, respectively [35, 48]. The diacylglycerol also served as a precursor for the "resynthesis" of the phosphoinositides, thereby completing a cycle referred to as the "PI Cycle." Furthermore, since phosphoinositides (PIs) often contain arachidonic acid esterified at the *sn*-2 position, hydrolysis of the resulting diacylglycerol would liberate this lipid for the production of biologically important eicosanoids. While this multifunctional role of PI hydrolysis provided an attractive hypothesis for lipid-mediated signal transduction, data from our laboratory, as well as others, demonstrated that another lipid, phosphatidylcholine (PC), was also hydrolyzed. In fact, PC hydrolysis contributed most, if not all, of the induced diglycerides in many systems [13, 18, 27, 52, 53, 56]. Furthermore, in most instances, phosphoinositide (PI) hydrolysis appears to be transient while PC hydrolysis was sustained. In some cases, PC hydrolysis occurs in the complete absence of an observable PI hydrolysis ([13, 18, 27, 52, 53, 56] and references therein).

It was previously assumed that agonist-induced phospholipid hydrolysis, resulting in increased levels of diglycerides, occurred at the plasma membrane. Quantification of the mass of induced diglyceride made it clear, however, that all of the induced diglyceride could not be present in the plasma membrane. In vasopressin-stimulated hepatocytes [3, 18], and α-thrombin-stimulated fibroblasts [52, 53, 66], the total amount of induced diglyceride represents nearly 1% of the total mass of cellular lipid. If all of this diglyceride was present in the plasma membrane, it would result in drastic and detrimental changes in the physical properties of this structure (e.g., [58]). In addition, Martin et al. [41] demonstrated that in GH_3 cells stimulated with thyroid releasing hormone (TRH), the small initial diglyceride production (possibly derived from PIPs) occurs at the plasma membrane while the larger later phase of diglyceride production (likely derived from PC) is produced in internal membrane. These data demonstrate that agonist-induced diglyceride production must occur at sites other than the plasma membrane.

In view of the fact that any signal transduction mechanism must account for the modulation of nuclear events (*see above*) and as a result of the data summarized above, the effect of agonist on nuclear diglycerides became an important question. One indication that these lipids may be elevated in nuclei was derived from studies of the localization of PKC. Using immunological and biochemical analyses, we and others have demonstrated the localization of PKC in the nucleus of IIC9 fibroblasts [33], liver [42], HL-60 cells [22], and 3T3 cells [10, 16, 20, 32, 62]. While in some systems, such as liver [42], PKC appears to be constitutively expressed in the nucleus, in other systems PKC is present in nuclei prepared from agonists stimulated, but not unstimulated, cells [10, 16, 20, 32, 33, 62]. Since PKC is a physiological target for diglycerides [48], these data suggest the hypothesis that nuclear association of PKC correlates with agonist-induced elevation of nuclear diglycerides.

The above hypothesis could be directly tested by determining the effect of agonist stimulation on the mass level of nuclear diglycerides. Indeed, we found a robust, rapid albeit transient rise in nuclear diglycerides in response to α-thrombin in quiescent IIC9 cells ([33] and Fig. 1). EGF also increases nuclear diglyceride levels (M.B. Jarpe, K.L. Leach and D.M. Raben, *unpublished observation*). The thrombin-induced rise was accompanied by an increase in the level of nuclear PKC-α [33]. These data are consistent with other reports demonstrating an IGF-1-induced increase in nuclear diglycerides and a concomitant increase in nuclear PKC activity in Swiss 3T3 cells [8, 10, 11, 16, 39]. These data clearly suggest a linkage between mitogen-induced nuclear lipid metabolism, PKC activation, and cellular proliferation.

It is important to note that it is difficult to compare the IIC9 studies with the 3T3 studies. In the 3T3 studies, nuclei were prepared in the presence of detergents while in the IIC9 studies, nuclei were isolated without detergents. The nuclei isolated in the 3T3 studies lacked at least an outer nuclear envelope which could result in a loss of nuclear diglycerides. Examination of IIC9 nuclei isolated in the absence of detergents shows that they possess intact inner and outer membranes [33]. Interestingly, if IIC9 nuclei are isolated in the presence of detergent, neither diglycerides nor PKC activity can be detected at any time (M.B. Jarpe, K.L. Leach, and D.M. Raben, *unpublished observations*). In contrast, induced increases in nuclear diglycerides cannot be detected when 3T3 nuclei are prepared in the absence of detergent, while this is required for the detection of nuclear PKC [16]. While the reasons for the differences in the two systems are unclear, they may reflect basic differences between the two cell types or purity of the nuclei prepared under the different conditions. This issue notwithstanding, in both studies the increase in nuclear diglycerides was rapid and mitogen dependent indicating that this response is one of the immediate agonist-induced nuclear responses.

Source of the Induced Nuclear Diglycerides

To identify the enzymatic mechanisms responsible for the increase in the induced nuclear diglycerides, it is critical to identify the lipids that serve as the source of those diglycerides. In the 3T3 cell studies, a small decrease in PIP and PIP_2 levels was observed, suggesting that the hydrolysis of PIs was responsible for at least part of the increase in IGF-1-induced nuclear diglycerides [16, 17]. The decrease in nuclear PIs, however, did not quantitatively account for all of the induced diglycerides [8, 10–12, 17], supporting the idea of another phospholipid source. In addition, since these nuclei were isolated in the presence of detergents (*see above*), examination of other potential sources, and complete quantification of all nuclear diglycerides, was not possible.

In the IIC9 cell studies we examined the source of nuclear diglycerides. First, we took advantage of our previous observation indicating that [^{3}H]myristate is preferentially incorporated into PC when intact IIC9s are acutely labeled [67]. An increase in radiolabeled nuclear diglycerides was observed after α-thrombin stimulation, suggesting that PC hydrolysis was a contributing source. However, differences in the temporal increases in diglyceride mass *vs.* radiolabeled diglycerides precluded a definite identification of all phospholipid sources [33].

Since it is not possible to selectively radiolabel nuclear phospholipids, potential sources of the induced nuclear diglycerides cannot be obtained by analysis of the release of water-soluble radiolabeled headgroups from metabolically labeled cultures. However, we have used an alternate method of determining the source of the induced diglycerides. In this technique, the molecular species of the induced diglycerides is compared with the molecular species of the potential phospholipid sources [53]. This analysis has proven useful in establishing the source of diglycerides generated in response to mitogens in fibroblasts [52, 53] and in response to neurotransmitters and neurotrophic factors in PC12 cells [51]. In addition, similar analyses have been used to identify PC as the source of induced diglycerides and PA in a variety of systems [1, 23, 34].

This molecular species analysis was applied to diglycerides generated in the nucleus of α-thrombin-stimulated IIC9 cells [25]. An analysis of the induced nuclear diglycerides indicates that PC hydrolysis is the predominant, if not exclusive, source of the induced diglycerides at all times. This was a surprising and interesting result since it was known that diglycerides generated in intact IIC9 cells are derived from two sources in response to a high concentration of α-thrombin [52, 53, 66]. Phosphoinositides are the primary source during the first 15–60 sec of stimulation while PC is the primary, if not exclusive source after 5 min [52, 53]. We should note, however, that small increases in nuclear PI-derived diglycerides, representing $\leq 1\%$ of the total nuclear lipid may have escaped detection.

In view of our data with the IIC9 cells, in addition to the studies with 3T3 cells, it is tempting to speculate that mitogens activate a PC cycle in the nuclear envelope as well as a PI cycle. In support of this hypothesis, enzymes involved in these cycles have been identified in the nucleus. PI cycle enzymes, PI-PLC, diglyceride kinase, and PI 4 and 5 kinases, have been localized in the nucleus [50]. We should note that a PI-3-kinase, which phosphorylates myo-inositol at the D-3 ring position and has been implicated as an important signal transduction activity [49], has not been identified in the nucleus. An enzyme involved in PC biosynthesis, CTP:phosphocholine cytidylyltransferase, has also been localized in the nucleus [64]. This enzyme is particularly interesting as it often serves as the regulatory enzyme in PC biosynthesis and its activity is regulated by diacylglycerol [28, 30]. In preliminary studies, a PC hydrolyzing activity has also been identified in isolated IIC9 nuclei (M.B. Jarpe and D.M. Raben, *unpublished observations*). These data provide strong support for the hypothesis that mitogens activate a PI and/or PC cycle in the nuclear envelope. While these data also argue that the nuclear diglycerides are derived from phospholipids in the nuclear envelope, we cannot completely rule out the possibility that at least some diglyceride may be generated in a non-nuclear membrane and then transferred to the nucleus.

Other Phospholipid Metabolism

In intact cells, the preponderance of data regarding agonist-induced phosphoglyceride metabolism has focused

on the metabolism of PIs and PC. There is compelling evidence, however, to implicate phosphatidylethanolamine (PE) metabolism in some systems. For example, phorbol esters have been shown to stimulate a PE hydrolysis, possibly via PLD-type enzyme, in certain cell types [29]. Plasmalogen PEs, PEs with a vinyl ether linkage at *sn*-1, have also been implicated as important stores for arachidonic acid released in response to some agonists [21].

In this regard, it is interesting that while the molecular species of whole-cell phospholipids does not change significantly in response to α-thrombin [52, 53], the molecular species of nuclear PE was dramatically altered. To begin with, nuclear PE profiles from quiescent cells had very little resemblance to whole-cell PE profiles [25, 52, 53]. Quiescent nuclear PE is composed predominantly of 16:0–18:1ω9 and 16:0–16:1ω7 [25]. Five minutes after the addition of α-thrombin, several species with later retention times were found, with a corresponding loss of the above-mentioned species with earlier retention times [25]. These data suggest that nuclear PE metabolism may also play a role in modulating nuclear events.

Arachidonic Acid Release and Metabolism at the Nuclear Envelope

The metabolism of arachidonic acid has been intensely studied. Much of this is due to the fact that metabolites of this fatty acid, collectively known as eicosanoids, represent an important and powerful class of second messengers. These eicosanoids, as well as unesterified free arachidonate and other unsaturated fatty acids, serve as intracellular or intercellular (autocoids) messengers which modulate a variety of cellular activities including the induction of specific genes and mitogenesis ([60] and references therein).

Due to the importance of arachidonic acid as a second messenger, much work has centered around the mechanisms responsible for the agonist-induced production of this lipid and its metabolites. There are two levels of regulation responsible for the production of these messengers. First, as almost all of the arachidonic acid in unstimulated cells is found esterified to phospholipids, the regulated release of this lipid represents the first level of regulation. It is now well recognized that two principal mechanisms exist for this release—hydrolysis from intact phospholipids via a phospholipase A_2-type enzyme, or the generation of diacylglycerol from a phospholipid, generally via a PLC-type enzyme, followed by hydrolysis of the diacylglycerol thus generating arachidonic acid. Second, the enzymes responsible for the metabolism of the released arachidonic acid may also be regulated and a number of excellent reviews exist on these topics [15, 60].

Less is known about the subcellular localization of the induced release and metabolism of arachidonic acid. Some of the arachidonic acid may be released from phospholipids in the plasma membrane and subsequently transported to intracellular membranes for metabolism. There is compelling evidence, however, that arachidonic acid may be released and metabolized in the nuclear envelope. Neufeld et al. demonstrated that arachidonic acid is preferentially incorporated into the nuclear membrane of a mouse fibrosarcoma cell line. Additional studies suggest that these diglycerides serve as the most important source of arachidonic acid which is released and converted into eicosanoids in response to bradykinin [31, 45, 46]. Similarly, Capriotti et al. demonstrated that in mouse fibroblasts the nucleus was the site where [^{14}C]arachidonic acid was most rapidly incorporated and provided the major source of radiolabeled eicosanoids released in response to bradykinin [69]. There is now convincing evidence demonstrating the presence in the nucleus of phospholipase A_2, cycloxygenases, 5 and 12 lipoxygenases, cytochrome P450 (in addition to related enzymes cytochrome oxidases 1 and 2) [9, 44, 61, 65]. These enzymes are involved in the release and metabolism of arachidonic acid providing strong support for the hypothesis that agonists induce this event at the nucleus.

Consequences of Nuclear Lipid Metabolism: The Biology

Physiological Role of Induced Nuclear Diglycerides

The above data provide strong support for the hypothesis that modulation of nuclear diglyceride and fatty acid levels are important components of mitogenic signal transduction pathways. In further support of this hypothesis, nuclei isolated from proliferating cells in regenerating liver also contain increased levels of nuclear diglycerides [2]. In addition, IGF-1 does not induce nuclear PKC in mutant 3T3 cells that contain IGF-1 receptors but fail to respond mitogenically to IGF-1 [40]. It would be interesting to examine the effect of IGF-1 on nuclear diglycerides in these mutants cells. If they are not elevated in response to IGF-1, it would provide further support for the hypothesis that these lipids modulate nuclear PKC activity. If they are elevated, it would suggest that the elevation of nuclear diglycerides is not a sufficient signal for the increase in nuclear PKC activity and/or serves other physiological roles.

One likely role for the induced nuclear diglycerides, as mentioned, is to mediate the activation of nuclear PKC. In this regard, it is interesting that mitogen stimulation of quiescent cells results in the phosphorylation of specific nuclear proteins [33, 43] and a number of

studies have provided compelling evidence implicating a role for the activation of nuclear PKC in agonist-induced changes in nuclear functions [5, 6, 16, 19, 22, 24, 32, 33, 36, 39, 42, 57, 62]. It is tempting to speculate, therefore, that the mitogen-induced nuclear diglycerides lead to the activation of nuclear PKC activity which is responsible for at least part of the mitogen-induced phosphorylation of selected nuclear proteins. It should be noted, however, that the precise relationship between these events has not been firmly established.

While the early whole-cell studies illuminated the central role of diglycerides in the activation of PKC and identified many of the important implications of this event, it has led many investigators to assume that the activation of PKC is the *only* biological effect of these lipids. There is substantial evidence that is not the case. In addition to these lipids being a major precursor of phospholipids and triglycerides synthesized *de novo,* diglycerides also modulate other cellular processes independent of PKC activation. For example, phospholipase activities [68], enzymes involved in lipid synthesis [28, 30, 64], the generation of superoxide in neutrophils [63], membrane fusion [58], and membrane/cytoskeletal interactions [4] may be modulated by diglycerides via PKC-independent mechanisms. It is not unreasonable to suspect that many biologically important consequences of elevated diglyceride levels remain to be determined.

Physiological Role of Phospholipid Metabolism

Other than providing a source of induced diglycerides, the role of phospholipid metabolism in the nucleus is largely an unexplored area. In addition to providing a source of nuclear diglycerides, other roles for nuclear phospholipids have been suggested. Phospholipid/chromatin interactions have been implicated in the regulation of gene expression [7, 37]. For example, in isolated nuclei, negatively charged phospholipids such as PI stimulate RNA polymerase, while neutral phospholipids, including PC, inhibit this activity [7, 37]. It is interesting in this regard that different phospholipids may have different effects on certain physical parameters, such as thermal stability and ultrastructure of DNA and chromatin, which may ultimately affect nuclear activities such as transcription and ribonuclear protein processing [7, 37].

Physiological Role of Nuclear Arachidonic Acid Release and Metabolism

The physiological significance of nuclear arachidonate release and metabolism is as yet undefined. In view that PKC has been localized in the nucleus and that some isozymes of PKC are modulated by arachidonate [48], it is tempting to speculate that at least one of the roles of released arachidonic acid is to activate nuclear PKC. In addition to PKC and PLA_2 itself, other nuclear enzymes involved in signal transduction cascades may be modulated by arachidonic acid or its metabolites. For example, arachidonic acid is known to modulate a number of activities other than PKC including Ca^{2+}/calmodulin-dependent protein kinase which has been found in the nucleus [54, 60].

Future Directions

Evidence in support of the NEST hypothesis that agonist-induced nuclear lipid metabolism represents an important component of signal transduction pathways is increasing. Clearly, further studies are necessary to test this rather provocative hypothesis. Future directions will certainly include investigations into the mechanisms by which nuclear lipid metabolism is induced as a result of the activation of cell surface receptors, identification of all forms of nuclear lipid metabolism and their regulation, and elucidation of the functional consequences of this metabolism.

Coupling

The mechanism by which the nuclear phospholipases are activated as a result of agonist binding to cell surface receptors remains obscure. In view of the observation that the PI-PLC identified in the nucleus is the β isoform [38] which is known to be modulated by GTP-binding proteins [55], it seems likely that these proteins may be involved in regulating nuclear phospholipase activities. Consistent with this hypothesis, preliminary data from our laboratory indicate that a nonhydrolyzable analogue of GTP (GTPγS) stimulates nuclear PC hydrolyzing activities. In addition to this pathway, the ability and mechanism(s) by which tyrosine-kinase-containing receptors induce nuclear lipid metabolism have not been fully explored. It is interesting to speculate, however, that protein kinase cascades resulting in the activation of mitogen-activated protein kinases (MAP kinases) may be involved in mediating the PC hydrolysis induced by both G-protein-coupled receptors and tyrosine-kinase-containing receptors (*see* [14] for a recent review of these kinases). The pathways by which these plasma membrane receptors are coupled to nuclear lipid metabolism are the focus of some present research.

Other Nuclear Lipid Metabolism

Most of the current research has focused on the production of diglycerides, or the release and metabolism of arachidonic acid. The metabolism of other nuclear phospholipids, such as sphingomyelin, PE, and phosphatidylserine (PS) has been somewhat ignored. Our recent data

on alteration of nuclear PEs induced by α-thrombin in the molecular species, suggest that this response is physiologically relevant. The role of this molecular species change, however, is not clear at this time.

A role for the release and metabolism of nuclear arachidonic acid also remains a mystery. It is likely that a physiological role exists given the agonist-dependent nature of this event and the reported ability of arachidonate and its metabolites to modulate certain activities. It would be interesting, for example, if epoxyeicosatrienoic acids generated via the nuclear cytochrome P450 served as a reactive adduct for coupling this fatty acid or phospholipids containing this fatty acid (PE?) to chromatin. This remains an area for which more research is needed.

Direct Demonstration of Functional Consequences

Perhaps one of the most important areas of future research will focus on determining whether a direct demonstration of functional consequences of nuclear lipid metabolism can be established. How does the lipid metabolism affect transcription and/or translation? Is nuclear lipid metabolism involved in regulating DNA replication? Is nuclear lipid metabolism involved in modulating global nuclear events such as apoptosis, nuclear envelope breakdown, or reformation of the nuclear envelope via condensation of nuclear vesicles? With regard to the latter, Sullivan et al. recently published some intriguing data to implicate IP_3 receptors in this process [59].

Taken together, the above data suggest the following hypothesis. Agonist stimulation of G-protein-coupled receptors (GPCR) or tyrosine-kinase-containing receptors (TKR) results in the nuclear activation of either PC hydrolysis, or the hydrolysis of PI and PC. This activation may involve heterotrimeric GTP-binding proteins, small molecular weight monomeric GTP-binding proteins such as an ARF (ADP-ribosylation factor), and/or the MAP kinase cascade. In the studies of α-thrombin stimulation of IIC9 cells, PC serves as the predominant source of nuclear diglycerides. The PI-derived diglycerides may be generated on the innermost leaflet since they could be detected only in nuclei which had been detergent-stripped and lacked at least an outer nuclear membrane. These diglycerides may modulate nuclear activities by interacting with the nuclear lamina or chromatin. On the other hand, diglycerides derived from PC hydrolysis, detected in intact nuclei, may be generated throughout the nuclear envelope, including the outermost leaflet, and may serve to modulate activities at the outer leaflet as well as within the nucleus (Fig. 2).

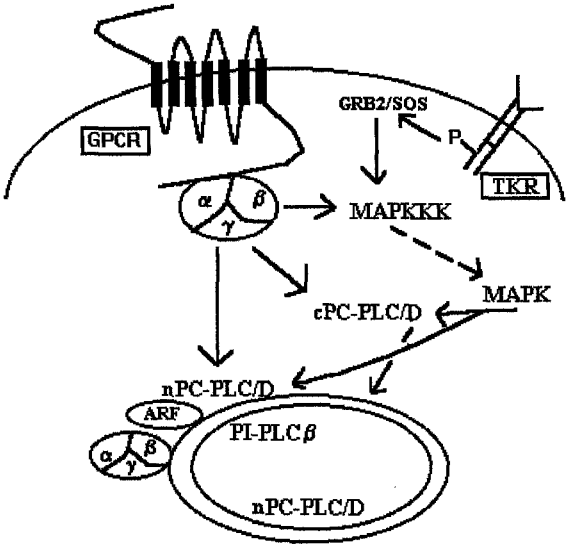

Fig. 2. Hypothetical model for agonist-induced nuclear PI and PC hydrolysis.

Summary

There is increasing evidence that nuclear lipid metabolism in NEST is an important new component in signal transducing networks and as a result, this metabolism is beginning to attract more attention. While agonist-induced nuclear lipid metabolism adds further complexity to the ever increasing array of signal transduction components, it also provides further avenues by which nuclear activities may be regulated. Identification of the coupling mechanisms, regulation, and physiological roles of nuclear lipid metabolism represents a new and exciting area of research which will have a broad impact in our understanding of signal transduction pathways.

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