Critical Review

Mechanistic Insights into the Nongenomic Regulation of Phospholipid Synthesizing Enzymes

Andrés M. Cardozo Gizzi
Beatriz L. Caputto*

Centro de Investigaciones en Química Biológica de Córdoba, CIQUIBIC (UNC-CONICET), Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, Córdoba, Argentina

Abstract

Lipid synthesis is a complex process regulated at multiple levels. Here, we will discuss nongenomic regulatory mechanisms, particularly the activation and/or recruitment of key enzymes to membranes. The phospholipid synthesis enzymes Lipin and CTP:phosphocholine cytidylyltransferase are taken as examples of these mechanisms that are mediated by post-translational modifications or by an intrinsic property of the enzyme that senses lipid composition. In addition, special emphasis will be put on another relevant non genomic lipid synthesis regulation mechanism that is dependent on c-Fos, a protein that has deserved less attention so far. This latter regulatory mechanism is emerging as an important determinant for processes that require high rates of lipid synthesis such as those of growth and proliferation. © 2013 IUBMB Life, 65(7):584–592, 2013

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Introduction

Membrane biogenesis is fundamental for cell growth and proliferation and for sustaining differentiation processes. Eukaryotic cell membranes are a complex mixture of glycerolipids, sphingolipids, proteins, and cholesterol whose assembly requires the synchronous activity of several metabolic pathways that are coordinated at multiple levels. Comprehensive reviews have focused on the genomic regulation of lipid synthesis (1,2); instead, we will focus this review on the modulation of enzyme activities via nongenomic regulation. Findings over the last few years are changing the perspective of lipid synthesis regulation. We will discuss in particular the activation and/or recruitment of key enzymes to membranes that results in regulation of their activity; giving special attention to c-Fos, which can act as an enzyme activator. To facilitate the reading, a schematic representation of the major pathways of phospholipid synthesis is included (Fig. 1).

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**Address for correspondence to: Beatriz L. Caputto, CIQUIBIC (UNC-CONICET), Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torres esq. M. Allende, Ciudad Universitaria, X5000HUA Córdoba, Argentina. Tel: 54-351-4334-074. Fax: +54-351-4334-074. E-mail: bcaputto@fcq.unc.edu.ar.*
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"Classic" Nongenomic Mechanisms

In the simplest mechanism, a reaction product inhibits its own production. This mechanism has been proven for phosphatidylserine synthase (PSS) 1 whose activity is inhibited by exogenously added phosphatidylserine (PtdSer). A R95K point mutated version of PSS1 has been shown to be resistant to inhibition by PtdSer in intact cells and in isolated membrane fractions, resulting in a ~2-fold increase both in the PtdSer biosynthetic rate and content (3). Also, cross regulation of lipid synthesis, namely the capacity of lipids of one particular pathway to positively affect the synthesis of a different metabolic branch, has been described. Phosphatidic acid (PA) sits at a key branching point where it can be diverted into the Kennedy

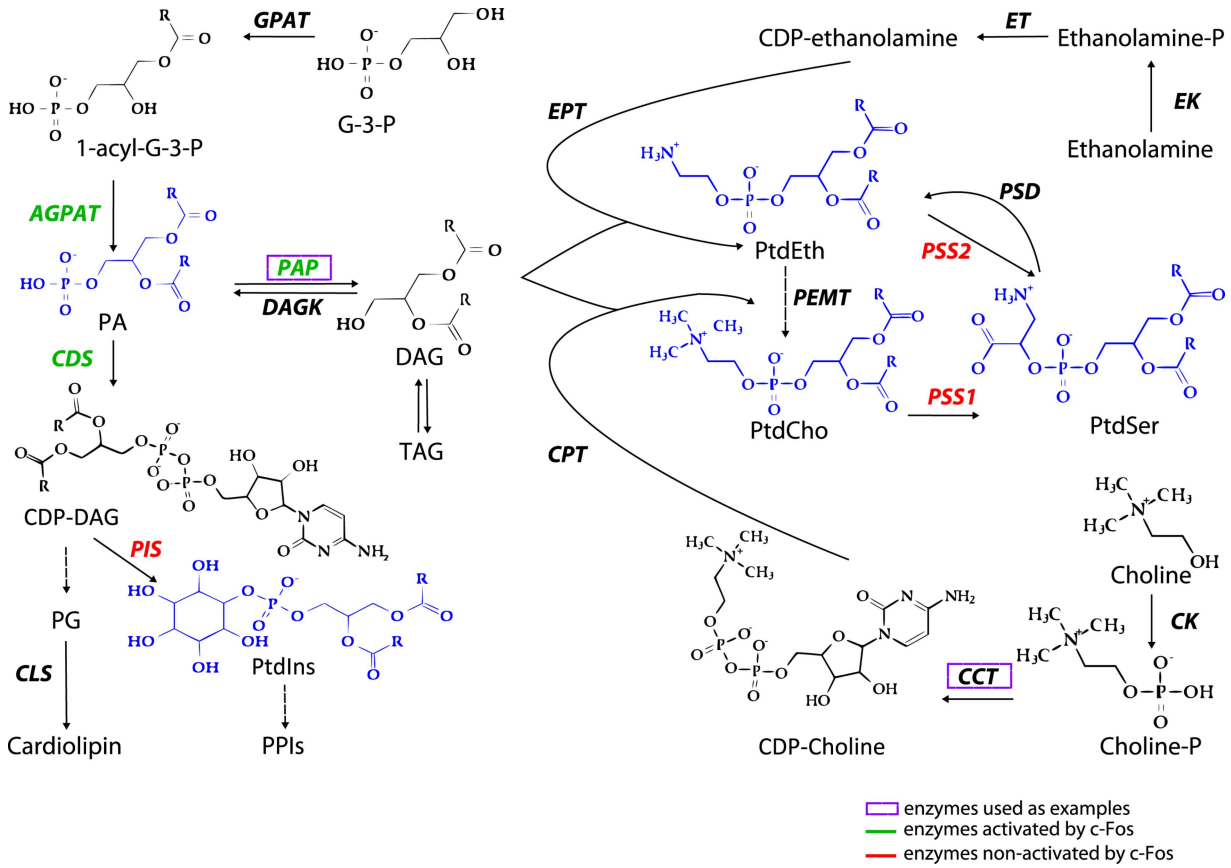

FIG 1

Pathways of glycerophospholipid biosynthesis in mammalian cells. G-3-P, glycerol-3-phosphate; 1-acyl-3-G-P, 1-acylglycerol-3-phosphate; PA, phosphatidic acid; DAG, diacylglycerol; CDP-DAG, cytidine diphosphate-diacylglycerol; PtdIns, phosphatidylinositol; PIPs, polyphosphoinositides; PG, phosphatidylglycerol; CL, cardiolipin; PtdEth, phosphatidylethanolamine; PtdSer, phosphatidylserine; PtdCho, phosphatidylcholine; P-Ethanolamine, phosphoethanolamine; CDP-Ethanolamine, cytidine diphosphate ethanolamine; P-Choline, phosphocholine; CDP-Choline, cytidine diphosphate choline; while R represents an alkyl chain of 14–24 carbons length, with 0–6 double bonds. Key metabolites are in blue. The enzymes are: GPAT, glycerol phosphate acyltransferase; AGPAT, acylglycerolphosphate acyltransferase; PAP, phosphatidic acid phosphatase; CDS, CDP-diacylglycerol synthase; PIS, phosphatidylinositol synthase; CLS, cardiolipin synthase; EPT, CDP-ethanolamine: diacylglycerol ethanolaminephosphotransferase; CPT, CDP-choline:diacylglycerol cholinephosphotransferase; EK, ethanolamine kinase; ET, CTP:phosphoethanolamine cytidylyltransferase; CK, choline kinase; CCT, CTP:phosphocholine cytidylyltransferase; PEMT, phosphatidylethanolamine N-methyltransferase; PSD, phosphatidylserine decarboxylase; PSS, phosphatidylserine synthase. It should be noted that c-Fos also activates PI4KIIα (not shown). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

pathway by a phosphatidate phosphohydrolase (PAP) or it can be used for the phosphatidylinositol (PtdIns) pathway of synthesis. The activity of the purified yeast PAP Pah1p, responsible for *de novo* dialcylglycerol (DAG) synthesis (4), is activated *in vitro* by CDP-DAG, PtdIns, and cardiolipin (5), a clear example in which the product/s of one pathway activate an enzyme of another one. Besides the transcriptional regulation and the mentioned "classic" nongenomic mechanisms, other mechanisms are now arising (6), including important theories on how membrane composition could regulate synthetic enzymes.

Superlattice Formation

According to the superlattice (SL) model of membrane organization, lipid components tend to adopt regular rather than random lateral distributions in the bilayer; only particular compositions could form such arrangements (7). Charge–charge repulsions, tighter packing of lipids and increase in rotational freedom (due to mixing of a lipid with a large head group with one of a small head group resulting in a decrease of big head group crowding), and the hydrophobic effect could explain the minimum free energy in a regular distribution. It should be underscored that despite the accumulating evidence, this is still an unproven theory. Although the arrangements are proposed as dynamic structures with a limited life-time, they could modulate the activity of certain surface-acting proteins. The SL-model predicts that when a lipid species exceeds the most favorable value/s, membrane packing defects appear because the molecules in excess cannot be accommodated. Several phospholipases are activated *in vitro* by these defects.

Also, abrupt changes in membrane packing upon SL formation could regulate synthetic enzymes that have the capacity to sense these changes.

Activity Regulation of Amphitropic Enzymes

An amphipathic helix (AH) motif present in a subset of amphitropic proteins can sense membrane quality and act as a membrane anchoring domain (8). CTP:phosphocholine cytidylyltransferase (CCT) is the regulatory enzyme for PtdCho biosynthesis, the most abundant phospholipid component of eukaryotic biomembranes (9). The AH of CCT is referred to M domain, comprising amino acids (AAs) 237–290 of rat CCT. Upon changes in the membrane composition, the M domain experiences a conformational change from a random coil into an alpha helix that simultaneously represses an autoinhibitory constraint and enables CCT to bind to membranes. In this way, it modulates its activity and maintains PtdCho homeostasis (10). Helix stabilization and membrane insertion are linked events: full catalytic potential of CCT is not achieved when its M domain is deleted but rather relies on membrane binding for the acquisition of an activating conformation (11). This conformational change involves a large increase in k_{cat} values and a 10-fold decrease in the K_m value for CTP (11,12), with no effect on the K_m value for phosphocholine.

CCT partitioning to membranes and subsequent activation is regulated mainly through the interaction of lipid modulators with its M domain. Several studies have identified diverse lipid activators normally classified as class I and class II. The first class of lipid activators include anionic lipids such as PA; its capacity to activate CCT binding activity is thought to be mediated by the negative charge of the lipid headgroup that could interact with positively charged AAs residues in the AH of CCT. On the other hand, class II lipids such as DAG or unsaturated phosphatidylethanolamine (PtdEth) share a common feature: increasing their content in a membrane tends to curl it toward the water (negative curvature). Because the bilayer prevents this tendency to curl, free energy is stored in the form of curvature elastic stress. When the content of class II lipids is relatively high, insertion of domain M could relieve the stored curvature stress by allowing nearby lipids to spread out. In this way, a purely physical feedback control mechanism could regulate the association/dissociation of CCT (13). So, membrane composition is again proposed to regulate enzyme activity.

During G_1, the rate of PtdCho catabolism is high but balanced by an increase in the activity of CCT. On the other hand, CCT overexpression leads to an increased CCT activity that is accompanied by an increased rate of PtdCho degradation by calcium independent phospholipase A_2 (14). Mechanisms involving activity regulation of these two enzymes through membrane composition could explain this interplay. The two already mentioned mechanisms (SL-formation and membrane curvature stress) are not mutually exclusive and perhaps both of them act coordinately. Downregulation of homeostatic phospholipases activity at the G1/S transition contributes to the accumulation of phospholipid mass in preparation for cell division (15).

The amphitropism of CCT involves more than just membrane partitioning; it also regulates its shuttling between the nuclear and endoplasmic reticulum (ER) compartments. The alpha isoform presents a nuclear localization signal (NLS) that confers a nuclear localization to CCT in many cell types (16). Still, activation of nuclear CCTα by anionic lipids results in nuclear export and requires membrane translocation and activation, which is also mediated by its M domain (17). Moreover, CCTα displays a stable cytosolic distribution in cells with an increased PtdCho demand such as pulmonary epithelial cells and tissues (18,19).

PtdCho is the precursor for PtdSer and sphingomyelin (SM). *Ergo*, the activity of CCT, not only regulates PtdCho content but also undoubtedly impacts on the rate of synthesis of these other lipids. Furthermore, CCT is activated by cholesterol, and therefore, cholesterol levels correlate with PtdCho synthesis (20). In fact, a reduced cellular cholesterol content prevents CCT activation and promotes a decrease in phospholipid content (21). These examples underscore that activity modulation of regulatory enzymes has profound consequences on the overall lipid content of a cell. For an extensive reading on CCT, the reader is referred to comprehensive reviews (16,22).

Another regulatory enzyme that has an AH motif is the PAP type I (the type I classification is due to its early biochemical characterization as Mg^{+2}-dependent). In yeast, there is one gene coding for PAP1 activity: p*ah1p*. Pah1p is recruited in a PA-dependent manner onto nuclear-ER membranes by means of the AH motif. Mutating the hydrophobic residues within this motif diminishes its affinity for substrate-containing micelle surface *in vitro*, which in turn results in reduced product formation (23).

In mammals, three genes encode for the Lipin family, the orthologs of Pah1p. They all share a NLS that is also a lipid binding motif with high selectivity for PA. The deletion or replacement of basic residues within the NLS motif of Lipin1 changes both its nuclear localization and its affinity for membranes (24,25). Ren et al. (25) have proposed a model in which the binding of Lipin NLS to PA antagonizes its nuclear import, thereby assigning a role to the NLS in sensing membrane PA content and regulating Lipin function. CCT also has a NLS with lipid binding capacity that acts in concert with its M domain (26).

Activity Adjustment via Posttranslational Mechanisms and Lipid Sensing

An additional mechanism to modulate enzyme activity is through posttranslational modifications that alter: (i) enzyme

activity, (ii) subcellular localization, and/or (iii) protein stability. Regulation by post-translational mechanisms and lipid sensing are not mutually exclusive events. In fact, they can function together as seen in the yeast Pah1p example: dephosphorylation of the enzyme's AH by the Nem1p-Spo7p complex enables it to associate to membranes (23). The mammalian Lipin 1 is phosphorylated in response to insulin through the mTOR signaling pathway, both in 3T3-L1 cells and primary rat adipocytes (27,28). Phosphorylation changes its membrane association capacity (preventing substrate accessibility) but this is not accompanied with activity modulation (28). In contrast, the mitotic phosphorylation of Lipin 1 and 2 in HeLa cells decreases not only the affinity of these enzymes to membranes but also their catalytic activity (29). Consequently, at least two phosphorylation responses are acting, although it remains to be established if different residues are being modified. Lastly, it has been observed that Lipin 1α is sumoylated *in vivo*, and this post-translational modification affects its nuclear localization. Mutating the two sumoylation consensus residues impairs its nuclear localization and consequently Lipin1α function as a transcriptional co-activator for MEF2 and PGC-1α (30). In conclusion, different levels of regulation can be acting on the same enzyme to modulate its activity.

So far, we have mentioned synthesis regulation by virtue of globally acting genomic mechanisms, or rapidly induced changes in enzyme activity via translocation or conformational changes. These rapidly induced changes are mediated by an intrinsic property of the protein that senses lipid composition, by posttranslational modifications or both. Another different, nongenomic lipid synthesis regulation mechanism is mediated by an immediate early gene (IEG) protein: c-Fos.

c-Fos is a Phospholipid Synthesis Activator

In its canonical function, c-Fos is a well-established transcription factor member of the AP-1 family. The rapid induction of c-Fos expression in response to extracellular stimuli is a feature of this protein and has been extensively documented (reviewed in 31,32). In this way, c-Fos transforms short-term stimuli, such as those promoted by growth factors, into long-term responses, such as cell proliferation. Early observations showed that the transcription and translation of c-Fos participates in the regulation of glycerolipid synthesis in the chick retina upon sensorial stimulation. The light/dark-promoted differences in phospholipid labeling are abolished in photoreceptor and ganglion cells when the expression of c-Fos is specifically inhibited (33,34). A schematic representation of c-Fos, c-Fos deletion mutants, and the c-Fos-related protein Fra-1 that will be discussed herein is shown in Fig. 2.

In quiescent fibroblasts induced to re-enter the cell cycle, c-Fos is rapidly and transiently induced and this correlates with two peaks of phospholipid synthesis activation; the first wave (at 7.5 min postinduction) affects predominantly the labeling of

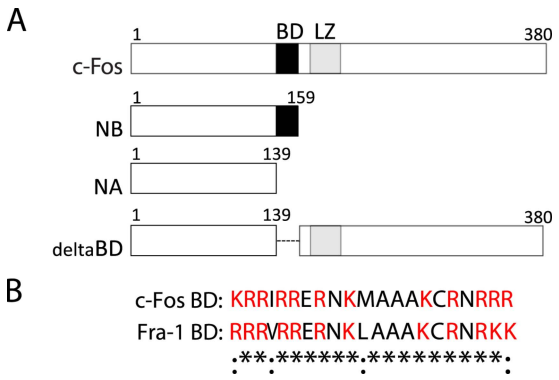

FIG 2

(a) Schematic representation of c-Fos and c-Fos mutants. (b) Aligned amino acid sequences of the BD of c-Fos and of Fra-1. The 12 basic amino acids are in red. "" Corresponds to conserved residue; ":" corresponds to conservation between groups of amino acids of strongly similar properties. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]*

polyphosphoinositides (PPIs), lipids whose regulated hydrolysis to generate second messengers has been well-characterized. The phospholipid species labeled during the second wave of activation (at 60 min postinduction) are more related to membrane biogenesis. Specifically blocking c-Fos expression returns phospholipid labeling back to control values. Interestingly, the inclusion in the cell culture medium of a nuclear localization signal peptide (NLSP) that impairs c-Fos nuclear import does not affect lipid synthesis activation, disclosing two c-Fos functions: one as a transcription factor in the nucleus and the other as a lipid synthesis activator in the cytoplasm (35).

Fos-dependent lipid activation has been observed in several cell types: decreasing c-Fos expression in human glioblastoma-derived T98G cells has the same decreasing effect on phospholipid labeling. Furthermore, inhibition of c-Fos-dependent glycerolipid synthesis activation also results in proliferation inhibition (Fig. 3) (36,38). Similar to what is seen in the G_0/G_1 transition of fibroblasts or T98G cells, c-Fos expression is rapidly upregulated after nerve growth factor (NGF) addition to PC12 cells, which triggers a genomic differentiation program that drives these cells into a sympathetic-neuron-like phenotype. At short times, cells need nuclear-localized c-Fos: NLSP treatment results in abrogation of neuritogenesis. However, after the first 16 h of NGF treatment, cells are already primed to grow and NLSP no longer affects neurite elongation. The presence of NGF also promotes a 2.5-fold increase in phospholipid labeling *in vivo* that is dependent on c-Fos expression. In primed cells, halting c-Fos expression determines neurite retraction even in the presence of NGF while overexpression of c-Fos alone sustains neurite outgrowth and elongation in the absence of NGF. In consequence, the genomic effect of c-Fos in initiating neurite outgrowth is distinct from its nongenomic effect of activating phospholipid synthesis to sustain elongation (39).

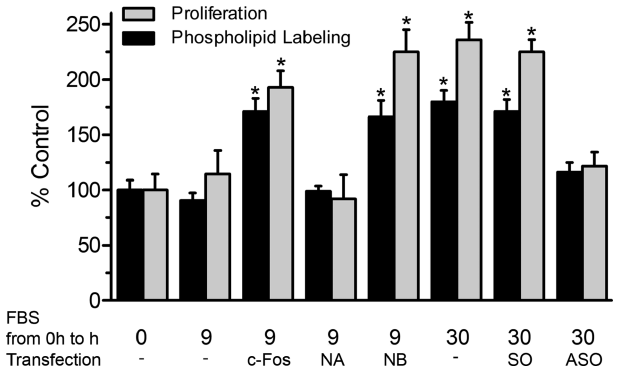

FIG 3

*T98G cells nontransfected or transfected with ASO, SO, c-Fos, or the indicated mutants were examined for phospholipid labeling (black columns) and for cell proliferation (gray columns). Quiescent cells were cultured +FBS for the indicated times, then medium was replaced for −FBS-medium and cultures continued to complete 30 h. ASO or SO were added to the medium 3 h before FBS addition. Phospholipid labeling was determined as described previously (34) after pulsing cells with 32P-orthophosphate during 2 h before harvesting at 30 h. Proliferation was determined at 30 h using the CyQUANT NF Cell Proliferation Assay Kit (Invitrogen). Results are the mean ± SD of three experiments performed in triplicate; *P < 0.002 with respect to column 1 as determined by one-way ANOVA with Dennett's post test. Figure adapted from (36,37) and our unpublished results. Note that only the phospholipid-activating proteins c-Fos and NB sustain proliferation after elimination of FBS at 9 h of culturing.*

Growth, proliferation, and/or differentiation in these models strictly correlates with the activated phospholipid synthesis state that relies on cytoplasmic c-Fos (Fig. 3). These particular examples point to cytoplasmic c-Fos as a shared mechanism to augment lipid supply and allows us to establish the following cause-consequence events: 1) c-Fos expression is rapidly induced by external cues, leading to 2) an increase in both AP-1 activity and phospholipid synthesis that, in turn, supports 3) cell proliferation and/or differentiation. The timing and localization of these two functions are different. Nuclear c-Fos is necessary at initial stages to elicit the corresponding genomic program, thus participating in priming cells to grow, whereas cytoplasmic c-Fos is necessary to support lipid synthesis both at initial and later stages of growth (36,37,39).

Cytoplasmic c-Fos in Brain Tumor Growth

c-Fos has been proven relevant in normal growth and differentiation processes and also as a potent oncoprotein when deregulated (40,41). Similar to what is seen in cultured brain tumor cell lines derived from a neuroblastoma (NB41A3), a glioma (C6), or a glioblastoma multiforme (U87M and T98G), c-Fos expression is required for tumor growth *in vivo* (37,42). All malignant human brain tumors examined (over 150) display abundant cytoplasmic c-Fos co-localizing with ER markers, which contrasts with normal brain samples that exhibit no detectable c-Fos expression. Brain tumor samples also have augmented total phospholipid *in vitro* labeling (42). Moreover, an intracranial xenograft of the T98G cell line into nude mice results in tumor development in 9 out of 10 animals treated with vehicle, in 7 out of 8 animals sense oligonucleotide (SO)-treated and in 0 out of 9 animals treated with antisense oligonucleotide (ASO) against c-Fos mRNA (37).

Another example of the *in vivo* relevance of c-Fos expression on brain tumor growth is found in NPcis animals, a mouse model of the human disease neurofibromatosis type 1, that spontaneously develop central and peripheral nervous system tumors. In the NPcis model, the previous observations hold true: there is an elevated c-Fos expression correlating with increased global phospholipid synthesis in the malignant tissue. Tumor progression is slowed down/stopped by treating tumors with ASO. Likewise, NPcis *fos* (−/−) mice show no tumor formation contrasting with a 74% prevalence in their littermates NPcis *fos* (+/+). It is worth underscoring that blocking c-Fos expression *in vivo* abrogates phospholipid synthesis activation without substantially affecting AP-1 content (42). These *in vivo* examples disclose c-Fos as a potential target to control brain cancer.

Molecular Mechanism of Lipid Synthesis Regulation by c-FOS

Regarding the mechanism of the general lipid synthesis activation by c-Fos, there is evidence it is achieved by increasing the activity of particular key enzymes that translocate to or are integral components of the ER. In the initial studies performed on retinal ganglion cells, higher PAP1 and 1-acylglycerol-3-phosphate acyltransferase activities were found in retinal ganglion cells from animals exposed to light (as compared with dark-maintained animals), whereas no dark/light differences were found in PSS 1 or PSS 2 activity. Treatment of retinas with c-Fos ASO abrogated the increase in enzyme activity (43).

Recombinant c-Fos activates *in vitro* CDP-diacylglycerol synthase (CDS) and PtdIns 4-kinase IIα, the first and third steps of PPIs synthesis, respectively (Fig. 1). However, no activation of PtdIns synthase (second step) or of PtdIns 4-kinase II β is observed. The V_{max} of the two activated enzymes are doubled, with no significant changes in the K_m upon c-Fos addition (44). This doubling in the V_{max} with no effect on the K_m is also observed for glycosphingolipid synthesis in differentiating PC12 cells. Specifically, c-Fos activates the enzyme glucosylceramide (GlcCer) synthase, the product of which, GlcCer, is the first glycosylated intermediate in the pathway of synthesis of glycolipids which leads to a global increment in ganglioside labeling. By contrast, the activities of GlcCer

galactosyltransferase 1 and lactosylceramide sialyltransferase 1 are essentially unaffected by c-Fos.

These findings support the notion that c-Fos is modulating only few steps of lipid synthesis to produce a general lipid synthesis activated state. Also, it seems that there is a shared activation mechanism in at least these two metabolic pathways. But how does c-Fos activate these enzymes? At early stages after stimulating cell cycle re-entry, c-Fos associates with components of the ER, as seen by confocal microscopy and differential centrifugation (35). Furthermore, c-Fos co-immunoprecipitates only with the enzymes whose activity it modulates in fibroblasts and differentiating PC12 cells (44,45). Besides, FRET (Förster Resonance Energy Transfer) microscopy ascertained a direct physical interaction between c-Fos and the enzymes it activates in the pathway of PPIs synthesis (44) (Fig. 4). Thus, a direct Fos/enzyme interaction at the ER seems a plausible mechanism for enzyme activation. Even when the predominant cellular localization is nuclear, monomeric c-Fos is actively undergoing nucleo-cytoplasmic shuttling (46). The reasons for this shuttling are not clear, but we hypothesize that the return of monomeric c-Fos to the cytoplasm is required to activate lipid synthesizing enzymes.

c-Fos Mutants Containing the Basic Domain (BD) Activate Phospholipid Synthesis

As an AP-1 transcription factor, c-Fos forms heterodimers with other members of the IEG family, mainly Jun, through a leucine-zipper (LZ) domain, whereas the AP-1/DNA interaction is accomplished through the BD domain (AAs 139–159). The BD domain of c-Fos has been shown relevant also for phospholipid synthesis activation. The sole addition of recombinant c-Fos or of particular c-Fos deletion mutants to quiescent cell homogenates augments *in vitro* phospholipid radiolabeling. This ~2-fold increment is attained only with the mutants containing the BD domain. Even the 21-mer BD peptide alone activates phospholipid synthesis, although a much higher molar concentration is required (39). Therefore, the BD region is necessary for both AP-1/DNA-binding activity and phospholipid synthesis activation.

When the ^{3}H-Glucose incorporation into endogenous glycolipid acceptors is tested *in vitro* the same is observed: the NB mutant (AAs 1–159, containing BD, see Fig. 2) but not the NA one (AAs 1–139, NOT containing BD) activates lipid synthesis (45). Furthermore, as occurs with full-length c-Fos, cells transfected to overexpress BD-containing mutants support neurite elongation in the absence of NGF (39). The independence of the genomic activity of c-Fos is clearly seen: the NB mutant that lacks the dimerization domain (and cannot form AP-1 dimers) is just as efficient as wt-c-Fos to activate lipid synthesis (Fig. 3). The relevance of the BD domain as a lipid activator has been further highlighted in Fra-1, another member of the *fos* family with a highly homologous BD domain (Fig. 2). Fra-1 also increases phospholipid synthesis and supports growth in breast cancer-derived cell lines and in human malignant breast tumors (47). Further work will be required to determine if there is a shared mechanism between c-Fos and Fra-1 to achieve lipid synthesis activation.

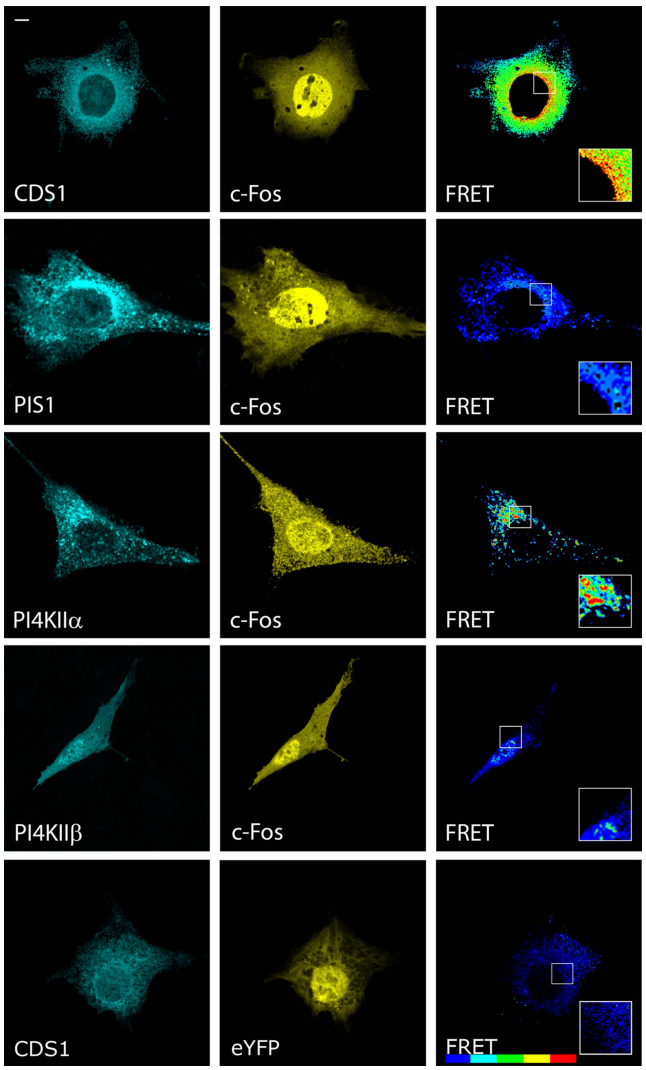

FIG 4

CDS and PI4KIIα but not PIS1 or PI4KIIβ undergo FRET with c-Fos. Cells cotransfected to express YFP-tagged c-Fos and the corresponding CFP-tagged enzyme were examined by confocal microscopy using filters for CFP (left) or for YFP (middle). FRET efficiency images were obtained and pseudocolored using PFRET software (right). The last row shows negative control cells co-expressing CDS1-CFP and YFP. Results obtained after the examination of 50 cells in each case are from one representative experiment out of three performed. Bar: 5 μm. FRET bar shown on the right corresponds to a blue-to-red increasing scale of FRET efficiency. Figure has been adapted from (44). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

N-Terminal Domain Mediates c-Fos Association to Enzymes

FRET microscopy also revealed that the mutants NA, NB, and deltaBD (wt-c-Fos except for the deletion of the BD domain) physically associate to CDS (44) and PI4KIIα (unpublished), the activated enzymes in the PPIs pathway of synthesis, irrespective of having the BD domain or not. The simplest explanation of these results is that c-Fos activates the enzymes through its BD domain, whereas binding is accomplished by its N-terminus. Further studies will establish if this binding segment is common to all the enzymes activated by c-Fos.

In line with the above observations, phosphorylation in the N-terminal region mediates c-Fos/ER association. Phosphorylation of c-Fos tyrosine residues 10 and 30 renders c-Fos unable to bind to the ER and hence to increase phospholipid synthesis. After mitogen stimulus of quiescent cells, c-Fos is rapidly expressed, dephosphorylated, membrane bound, and activating glycerolipid synthesis (36,38). These results support the notion that the N-terminal domain of c-Fos mediates its association to particular enzymes and that this association is ultimately regulated by the phosphorylation status of tyrosines 10 and 30.

c-Fos is a Surface Active Intrinsically Unstructured Protein (IUP)

How can c-Fos activate various enzymes that share no obvious similar structures? The answer might be found in the intrinsically disordered nature of c-Fos. It belongs to the family of IUPs, proteins with large regions that lack a well-defined 3D structure in their native state (48). c-Fos is predicted to have an overall disorder of 40–60% (depending on the algorithm used). The inherent nature of IUPs confers them two advantages: 1) their conformational plasticity allows them to recognize and bind different partners with high specificity and 2) the unique combination of high-specificity and low-affinity interactions (49).

Our evidence suggests that c-Fos binds to key enzymes via its N-terminal domain. However, it remains to be established if there is a disorder-to-order transition coupled to binding. Its BD domain has been proven to undergo a disorder-to-order transition upon DNA binding as an AP-1 complex, forming an alpha helix that interacts directly with DNA. We propose that the folding of c-Fos upon binding to its targets could be relevant for c-Fos/enzyme interaction.

Another characteristic determined by the IUP nature of c-Fos is its interfacial behavior. c-Fos is an amphitropic protein that by means of its flexible nature exerts surface activity. Biophysical studies have shown its high affinity for membranes and its capacity to sense and respond to changes in monolayer lipid composition and phase *in vitro* (50,51), supporting the idea that c-Fos has the adequate molecular features (high surface activity, lipid sensing, and intrinsic disorder that allows for promiscuity) (52) to perform a regulatory role in glycerolipid synthesis. On a cellular context, c-Fos can be envisaged sensing lipid composition, binding to ER membranes and finding its way to target enzymes to activate them.

Conclusions and Perspectives

The mentioned observations underscore c-Fos as a ubiquitous modulator of both phospholipid and glycolipid synthesis in proliferation and differentiation. We hypothesize that c-Fos-activated lipid synthesis is crucial to support membrane biogenesis demands required for these processes. Future studies will determine the precise events involved in c-Fos regulation (expression, posttranslational modifications, subcellular localization and interaction partners) that couple nuclear and metabolic responses to proliferation, differentiation, and growth demands.

Throughout the review, we have underlined what is known about the modulation of the activity of regulatory lipid synthesis enzymes. The regulatory roles of CCT and Lipin are well-established and lean on the capacity of these enzymes to modify their activity in response to both extracellular and intracellular cues. On the other hand, c-Fos is able to activate particular enzymes to accomplish an overall phospholipid synthesis activation. Unpublished results from our lab show that c-Fos is also modulating both CCT and Lipin activities, thus indicating that c-Fos regulates lipid synthesis in response to cell demands. The balance between regulation of enzymes by translocation/post-translational modifications during cellular proliferation or growth versus c-Fos-dependent regulation will, undoubtedly, be in the focus of future, exciting studies.

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