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Review

c-Fos: An AP-1 transcription factor with an additional cytoplasmic, non-genomic lipid synthesis activation capacity

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ABSTRACT

The mechanisms that co-ordinately activate lipid synthesis when high rates of membrane biogenesis are needed to support cell growth are largely unknown. c-Fos, a well known AP-1 transcription factor, has emerged as a unique protein with the capacity to associate to specific enzymes of the pathway of synthesis of phospholipids at the endoplasmic reticulum and activate their synthesis to accompany genomic decisions of growth. Herein, we discuss this cytoplasmic, non-genomic effect of c-Fos in the context of other mechanisms that have been proposed to regulate lipid synthesis.

1. Introduction

Lipids (phospholipids, glycolipids, cholesterol) are quantitatively important molecular species of cell membranes. Among the vast diversity of known lipids, glycerophospholipids [phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PtdIns), phosphatidylserine (PS) and cardiolipin] are the most abundant in eukaryote cell membranes. Although glycerophospholipids are classified according to the structure of their polar head group (ie. choline or ethanolamine or inositol, etc.), each class of phospholipid in turn, consists of numerous molecular species that contain the same head group but differ in the acyl chains that each one contains. So, hundreds of different glycerophospholipid molecules together with sphingolipids, sterols and proteins, form the complex membranes of eukaryote cells. To reach this complexity, eukaryotic cells need to invest substantial resources: approximately 5% of their genes are involved in the synthesis of lipids [1,2].

Membrane biogenesis is a complex process that couples nuclear responses to growing environmental cues with appropriate morphological and functional changes of the cell. The proteins and lipids required for cell membrane expansion, i.e. during cell proliferation, neuritogenesis, tumorigenesis, etc. are provided by the endomembrane system, particularly the endoplasmic reticulum (ER) and the Golgi complex. Phospholipids, together with cholesterol and integral membrane proteins, are synthesized in the ER and incorporated into preexisting membrane. Nascent membranes bud at ER exit sites and move by vesicular transport toward the plasma membrane passing through the Golgi complex where a series of post-translational modifications on cargo and membrane-bound proteins occur. The lipid composition of membranes is also adjusted in the Golgi complex by the addition of glycolipids and sphingomyelin. In addition, non-vesicular transport has an important role in intracellular lipid trafficking and distribution. Monomeric lipid exchange, either spontaneous (slow) or mediated through lipid-transfer proteins (much faster), is greatly enhanced by membrane contact sites, defined as small cytosolic gaps between the ER and practically all the other cellular organelles [3].

Cells that are actively involved in proliferation or in events of plasma membrane expansion demand massive membrane biogenesis; so, it is reasonable to expect organelle homeostasis to be different to that of cells that are neither dividing nor actively growing. However, the nature of the regulatory events that control such processes is still poorly understood. From a simplistic point of view, the mechanisms that regulate lipid synthesis can be divided into two broad groups: one at the level of transcription and translation of the involved enzymes (genomic regulation), and the other at the post-translational level (non-genomic regulation). Although genomic regulation is of paramount importance for cell development, intuitively, it appears difficult to envisage that

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PtdIns, phosphatidylinositol; PS, phosphatidylserine; ER, endoplasmic reticulum; PSS, phosphatidylserine synthase; CCT, CTP:phosphocholine cytidylyltransferase; GPAT, Acyl-CoA:glycerol-3-phosphate acyltransferases; AP-1, Activator Protein-1; CNS, central nervous system; PNS, peripheral nervous system; ASO, antisense oligonucleotide; NLSP, Nuclear Localization Signal Peptide; NGF, nerve growth factor; LZ, leucine zipper; BD, basic domain; PPIs, polyphosphoinositides lipids; CDS, CDP-diacylglycerol synthase; PIS, phosphatidylinositol synthase; PI4KII, phosphatidylinositol-4-kinase type II; GlcCer, glucosylceramide; PAP1, phosphatidate phosphatase-1; AGPAT, 1-acylglycerol-3-phosphate O-acyltransferase; FRET, Förster Resonance Energy Transfer

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this is the mechanism that evolved in cells to assure the rapid response required to fulfill cellular membrane demands in response to environmental cues sensed by the cells such as growth factors, hormones, and neurotransmitters. Rather, diverse non-genomic regulatory mechanisms appear at first sight as faster responses than the genomic ones.

2. Non-genomic regulation of enzyme activity

The mechanisms underlying the regulation of the rate-limiting enzymes of phospholipid biosynthesis have not yet been established. However, in principle, several strategies, operating alone or coordinately, could be involved. The regulation imposed by the amount of a particular lipid that a membrane contains is one of these regulatory mechanisms. PS, the product of phosphatidylserine synthase 1 (PSS1), is an inhibitor of the enzyme's activity and is therefore commonly considered as a "product inhibition" mechanism [4]. More globally, membrane status also regulates enzyme activity. Such is the case observed upon the "membrane elastic stress" that is generated when the increased content of lipids that induce negative curvature (i.e. DAG) generates a curvature tension. CTP:Phosphocholine Cytidylyltransferase (CCT), the rate-limiting enzyme in PC synthesis, is able to sense this curvature stress, insert into the membrane and relax it while releasing an auto-inhibitory constraint on the catalytic site of the enzyme [5]. Increased DAG production that accompanies G_0 to G_1 transition has been proposed as responsible for CCT translocation to membranes and subsequent activation [6]. If attention is put on the protein side rather than on lipids, it has also been shown that post-translational modifications of lipid synthesis enzymes can dramatically change their activity. Insulin generates a phosphorylation cascade that leads to the phosphorylation of Acyl-CoA:glycerol-3-phosphate acyltransferases (GPATs) 3 and 4 that up-regulates their activity [7]. Insulin also promotes phosphorylation of the phosphatidic acid phosphatase Lipin, but in this case, phosphorylation down-regulates its association with membranes and consequently its activity [8]. In summary, external signals and internal cues regulate key enzymes with the capacity to sense these changing environmental conditions. In this complex scenario, we would like to discuss an additional regulatory mechanism that is mediated by the protein c-Fos, which is emerging as a shared regulatory mechanism for different enzymes in many cell types.

3. c-Fos-dependent phospholipid synthesis regulation

c-Fos was first described as a member of the AP-1 family of inducible transcription factors more than 25 years ago [9]. The cellular content of c-Fos is tightly regulated: this protein is at the limit of detection in quiescent cells, whereas its expression is rapidly but only transiently induced as part of the nuclear response to a plethora of stimuli such as growth factors, sensorial stimulation, and neurotransmitter release [9–12]. The first report showing that in rat spinal neurons c-Fos is induced shortly after sensorial stimulation, was by Hunt and colleagues in 1987 [13]. Thereafter, many reports provided evidences that both physiological and pathological conditions are capable of inducing the expression of c-Fos and of other members of this family of inducible transcription factors in the central nervous system (CNS) and in other cell types. Newly formed c-Fos participates in the formation of a diversity of active heterodimers with other proteins that belong to this family of inducible transcription factors, mainly with the protein c-Jun. These dimers constitute the highly charged, basic DNA-binding transcription factors termed Activator Protein-1 (AP-1). AP-1 activity has been involved in transmitting short-termed, growth-promoting signals into longer lasting changes by regulating the expression of target genes involved in cell growth such as collagenase [12,14], stromelysin [15], and metallothionein IIA [16]. When the corresponding stimulus ceases, c-Fos is rapidly degraded having a half life in the minute to the hour range, according to the cell type [17]. Nevertheless, the cellular consequences of inducing c-Fos expression together with the molecular mechanisms in which this protein participates are still being unraveled. Work from our lab has established that c-Fos is a moonlighting protein (a protein with more than one, apparently unrelated, function) capable of regulating growth not only by its transcription-factor activity but also because it can act as a cytoplasmic activator of the biosynthesis of lipids both in normal and pathological cellular processes that demand high rates of membrane biogenesis. The intrinsically disordered nature of c-Fos and the structural malleability that this entails can in principle explain the ability to perform both functions, as has already been proposed for intrinsically unstructured proteins [18] and particularly for c-Fos [19].

4. Cellular events in which c-Fos activates lipid synthesis

c-Fos-dependent activation of lipid synthesis has been observed in several cell types: in vivo in light-stimulated retina ganglion and photoreceptor cells [20,21], in growing NIH 3T3 cells [22], in PC12 cells induced to differentiate [23,24], in tumors of both the CNS and peripheral nervous system (PNS) [25,26] and in malignant human mammary tumors [27].

In retina, a notable difference was found between the response of photoreceptor and ganglion cells when submitting chicks to light stimulation with respect to the synthesis of phospholipids and to the expression of c-Fos: both increase upon light stimulation in ganglion cells and decrease in the photoreceptor cells. Specifically blocking c-Fos expression also blocks light-induced modifications in the synthesis of phospholipids in both cell types [20,21]. Since depolarization for neurotransmitter release occurs in light in the ganglion cells but in the dark in the photoreceptor cells, it was interpreted that the burst in c-Fos expression responds to the cell's need of increasing the rate of membrane biogenesis to replenish synaptic vesicles recycling upon neurotransmitter release [21].

In NIH 3T3 cells induced to re-enter the cell cycle, two waves of c-Fos expression promote concomitant waves of stimulated incorporation of ^{32}P-orthophosphate into phospholipids. The first wave of c-Fos expression peaks at 7.5 min and returns to control levels by 15 min; the second one starts by 30 min and remains elevated at least up to 120 min, the longest time examined. The lipids that incorporate ^{32}P during the first wave are predominantly second-messenger polyphosphoinositides lipids (PPIs) whereas in the second wave, membrane biogenesis-related lipids are the major radioactive products. The half-life of c-Fos mRNA is very short in this first wave, of only 10 min, while it is of 85 min in the second one [22].

Membrane biogenesis requires the coordinated supply of its various integral components. In this regard it was noticeable that in the retina ganglion cells, isotopic labeling experiments performed both in vivo (^{3}H-glycerol, ^{32}P-orthophosphate acid) and in vitro (^{32}P-γ-ATP), consistently showed all the labeled phospholipids to be similarly increased in a c-Fos-dependent manner in light with respect to dark [20,28]. Similar results were found in PC12 cells induced to differentiate to sympathetic-like neurons with NGF (nerve growth factor): c-Fos activates the overall metabolic labeling of both phospholipids [23] and glycolipids [24]. Blocking c-Fos expression impairs both neuritogenesis and the activation of phospholipid and glycolipid synthesis [23,24]. TLC analysis of total radioactivity in lipid extracts after metabolic labeling of these cells with [^{14}C]Gal or with ^{32}P-orthophosphate acid showed a 50–60% increase in the labeling of all ^{14}C-labeled glycolipids and of PC that is also labeled with [^{14}C]Gal and of all ^{32}P-labeled phospholipids. These results corroborate a global stimulation of the lipid-synthesizing machinery in the c-Fos-mediated response of PC12 cells to NGF [24].

At present there is abundant information regarding the genomic events that underlie uncontrolled, exacerbated growth of tumor cells [29]. However, reports on the pleiotropic changes that necessarily accompany tumor growth and proliferation are scarce. Even so, high rates of proliferation were found tightly coupled to an elevated expression of c-Fos together with activated rates of phospholipid synthesis in

different tumor models. Specifically blocking c-Fos-activated phospholipid synthesis significantly reduces the in culture proliferation of T98G cells, which derive from a human glioblastoma multiforme tumor. Furthermore, in nude mice xeno-grafted intracranially with T98G cells, treatment of animals at the engraftment site with c-Fos mRNA antisense oligonucleotide (ASO) impairs tumor growth that otherwise develops in near 90% of control animals. Similar results were found in NPcis mice, an animal model of the human disease Neurofibromatosis Type I that spontaneously develops CNS and PNS tumors: these tumors grew significantly slower than in controls when NPcis mice bearing tumors of 600 mm^3 were treated with c-Fos ASO and tumor volume measured over time. Further confirmation of the dependence of tumor growth on c-Fos expression was obtained with NPcis $Fos^{-/-}$ mice that do not develop tumors, which contrasts with the development of tumors in 71.4% of their NPcis $Fos^{+/+}$or $Fos^{+/-}$ littermates [25].

In summary, the data available at present allows us to hypothesize a shared activation mechanism in response to high-rate membrane biogenesis demands of cells irrespective of these demands arising from physiological or pathological situations as described herein. Not only tumor-associated pathologies show ER-associated c-Fos: in spinal cord of rats sensitized to develop experimental allergic encephalomyelitis in which positive reactive gliosis is promoted, c-Fos expression is also increased and is ER-associated [30]. Furthermore, the addition of c-Fos both to a non-pathological brain preparation obtained from a GM-patient as well as to the brain tumor sample stripped of associated proteins, results in an activated rate of phospholipid synthesis [25].

5. Is c-Fos-dependent lipid synthesis activation a genomic AP-1 phenomenon or is it a cytoplasmic event?

The observed effect of c-Fos on phospholipid synthesis could, in principle, be a consequence of its genomic AP-1 activity. However, an accumulating body of experimental evidence supports that the activation of lipid synthesis promoted by c-Fos is accomplished through a non-genomic mechanism as will now be summarized.

5.1. A dual function for c-Fos

c-Fos expression is rapidly up-regulated after addition of NGF to cultured PC12 cells which induces neuritogenesis. If together with NGF, cells are fed a Nuclear Localization Signal Peptide (NLSP) that blocks the import of AP-1 transcription factors into the nucleus, neuritogenesis is abrogated despite the fact that c-Fos is expressed normally. This indicates that AP-1-c-Fos must reach its nuclear localization to trigger neuritogenesis. Contrastingly, if NLSP is added 16 h after onset of NGF treatment, neurite elongation is not significantly impaired showing the importance of nuclear c-Fos-containing-AP-1 to initiate the genomic differentiation program. It also shows that nuclear c-Fos is not required to sustain neuritogenesis. However, if c-Fos expression is blocked once neuritogenesis is triggered (16 h after initiating NGF treatment), not only does the rate of phospholipid synthesis return to that of non-growing cells, but also cells halt growing and preformed neurites retract. This result indicates that extra-nuclear c-Fos is required to sustain growth.

In cells with blocked c-Fos expression, growth continues normally if primed cells are transfected to express c-Fos or a c-Fos deletion mutant such as c-Fos:1–160 (aa 1–160) that activates phospholipid and glycolipid synthesis in spite of the lack of the domain required for AP-1 dimmer formation (see 5.3). These results distinguish the dual function of c-Fos: it initially releases the genomic program for differentiation as an AP-1 transcription factor in the nucleus whereas it cytoplasmically activates phospholipid and glycolipid synthesis required for membrane genesis [23,24].

A similar experimental paradigm was used to examine c-Fos requirement to drive human brain tumor cell proliferation: T98G cells grown in proliferation medium show that at 30 h of cell priming (+FBS) in the absence of NLSP, the number of cells roughly doubles. Addition of NLSP to the culture medium at 0 or 6 h after FBS blocks cell proliferation whereas at 9 h it is no longer effective, indicating that nuclear AP-1-c-Fos is required to trigger proliferation only at early stages of cell proliferation. On the other hand, cytoplasmic c-Fos is required at all time points because blocking its expression by the addition of ASO to the culture medium at any time after feeding FBS blocks both proliferation and phospholipid synthesis activation [26].

5.2. In vitro activation of phospholipid synthesis by purified c-Fos

Direct confirmation that c-Fos affects the rate of phospholipid synthesis per se by a non-genomic mechanism was obtained by measuring the incorporation of ^{32}P from [^{32}P]-ATP into phospholipids in vitro in the presence or the absence of purified c-Fos or of its most common AP-1 dimer partner, c-Jun. Quiescent cell total homogenate or nuclei-free homogenate that contains negligible levels of endogenous c-Fos or c-Jun was used as the enzyme source. In both preparations, the addition of exogenous c-Fos to the assays activates phospholipid synthesis and does so in a concentration-dependent manner. Maximal activation is attained at 1 ng c-Fos/μg homogenate protein that corresponds to a concentration of ~10^5 molecules of c-Fos/cell [23]. This concentration of c-Fos is comparable with that calculated by Kovary and Bravo [12] to be contained by fibroblasts when endogenous c-Fos expression is induced (10^3–10^5 molecules of c-Fos/cell). c-Jun, that forms both homo- and hetero-dimer AP-1 complexes [31], had no effect on the rate of phospholipid synthesis when assayed at the same concentration as c-Fos or 10× the concentration required for c-Fos to promote maximum activation [32].

5.3. The BD domain of c-Fos is sufficient for lipid synthesis activation

Diverse c-Fos deletion mutants were tested in vitro for their capacity to activate phospholipid and glycolipid synthesis. Of special interest was the case of the mutant c-Fos:1–160 (aa 1–160) that contains the basic domain of c-Fos (aa 139–159) but lacks the domain required for AP-1 dimer formation, the leucine zipper (LZ) domain (aa 165–193). In spite of the fact that c-Fos:1–160 is unable to form AP-1 dimers, it is as effective as full-length c-Fos to activate glycolipid synthesis in vitro [24] and phospholipid synthesis both in vivo and in vitro [23]. In fact, all BD-containing mutants activate phospholipid synthesis whereas those that lack this domain (i.e. ΔBD that is full length c-Fos but only lacking the BD domain) fail to do so. Contrarily, the mutant c-Fos:165–380 that contains the LZ domain up to the C-terminus of c-Fos does not activate lipid synthesis [23,33].

Furthermore, Fra-1, a member of the Fos family of proteins that shares a highly conserved BD region with a difference of only two conservative substitutions, is also capable of increasing phospholipid synthesis and supporting growth of breast cancer-derived cell lines and of human malignant breast tumors [27].

6. Regulation of the capacity of c-Fos to activate lipid synthesis

Taken together, the results described above strongly support the notion that lipid synthesis activation mediated by c-Fos is achieved by a genomic-independent mechanism. If so, it seems reasonable to expect a rigorous control on the capacity of c-Fos to act both as a transcription factor and/or as a lipid synthesis-activator because of the importance it has on the metabolic outcome of a cell. Consequently, the next question posed was the molecular mechanism by which c-Fos exerts these capacities and how they are regulated.

As mentioned previously, one level of control of both its transcription factor activity and its lipid synthesis activating capacity is achieved by the precise control the cell imposes on its c-Fos content: both its expression and its degradation are tightly controlled [34]. Additionally, when acting as a transcription factor, its capacity to bind DNA is also

strictly controlled by several ser/thr kinases that act on c-Fos. In particular, ERK and RSK phosphorylate c-Fos within its C-terminal transactivation domain soon after growth-factor stimulus, leading to an increase both of the stability of c-Fos protein and of its transcription factor capacity [35,36].

Concerning the capacity of c-Fos to activate lipid synthesis in the cytoplasm, this requires the association of c-Fos to the ER [22], the quantitatively most important site of phospholipid synthesis in the cell. This association is regulated by the phosphorylation state of c-Fos but in this case on tyrosine residues rather than on serine residues. Quiescent cells contain very low amounts of c-Fos that, in addition, is tyr-phosphorylated, is not membrane bound and cells show basal levels of phospholipid synthesis. Inducing cells to grow promotes both abundant c-Fos expression and c-Fos dephosphorylation which results in its association to the ER membranes and in the activation of phospholipid synthesis [32]. c-Src was identified as the first kinase and TC45-PTP as the first phosphatase that phosphorylate and dephosphorylate tyr residues of c-Fos, respectively (schematized in Fig. 1A) [37]. Furthermore, when ^{32}P incorporation into phospholipids from [^{32}P]-ATP was measured in vitro, only dephosphorylated c-Fos was capable of activating phospholipid synthesis (Fig. 1B). Subcellular fractionation studies evidenced the non-membrane bound nature of phosphorylated c-Fos in opposition to the membrane bound state of dephosphorylated c-Fos [32].

The regulation of this reversible post-translational modification of c-Fos is imposed on the de-phosphorylation step rather than on the phosphorylation one. The induction of cells to re-enter growth with mitogens promotes the translocation of TC45-PTP from the nucleus to the cytoplasm, the activation of this PTP and the concomitant c-Fos/TC45 complex formation that results in c-Fos dephosphorylation. Moreover, knocking down TC45-PTP impairs both the dephosphorylation of c-Fos and the activation of lipid synthesis [37]. Many studies have shown the activation of c-Src upon cell stimulation (reviewed in [38]). However, no activation for the phosphorylation of c-Fos is seen when cells are induced to re-enter growth indicating that basal activity of c-Src is sufficient to maintain the small amounts of c-Fos contained in quiescent cells in its phosphorylated state [37].

It should be highlighted that tyr and ser/thre phosphorylation sites are eliciting opposed effects on the different activities of c-Fos: while the first one represses its non-genomic activity, the latter one activates its AP-1 activity.

It has been already proposed that the expression of c-Fos alone does not determine the biological outcome it promotes. Rather, it depends on the post-translational modifications that occur on c-Fos together with the duration and intensity of the stimulus imposed on the cell [36].

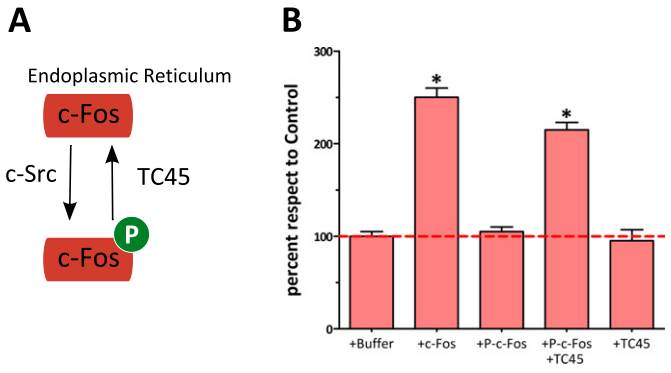

Fig. 1. Schematic representation of the tyrosine phosphorylation/de-phosphorylation cycle of c-Fos and its consequences on phospholipid synthesis activation. A. c-Fos is phosphorylated on tyrosine residues by the kinase c-Src and dephosphorylated by the phosphatase TC45-PTP. Note that dephosphorylated c-Fos is able to associate with the ER whereas phosphorylated c-Fos is not. B. Recombinant c-Fos was incubated in the absence (+c-Fos) or the presence (+P-c-Fos) of recombinant c-Src. Phosphorylated c-Fos (P-c-Fos) was then incubated with TC45 (+P-c-Fos/+TC45). A control with TC45 incubated alone was included (+TC45). Then, all incubates were assayed in vitro for their capacity to activate ^{32}P-phospholipid labeling from [^{32}P]-ATP. *$P < 0.002$. Note that only de-phosphorylated c-Fos activates ^{32}P incorporation into phospholipids. From Ferrero et al. [37].

7. c-Fos activates only particular enzymes of the pathway of synthesis of lipids

In the pathway of synthesis of PPIs, the in vitro activities of CDP-diacylglycerol synthase (CDS) and phosphatidylinositol-4-kinase type II (PI4KII) α (the first and third steps of the pathway) were activated by exogenous c-Fos. No activation of the second step, catalyzed by phosphatidylinositol synthase (PIS) or of PI4KII β (third step) was observed. Upon c-Fos addition, the V_{max} of the two activated enzymes doubled with no significant changes in the K_m [33].

The activation of particular metabolic steps in retinal ganglion cells of animals exposed to light with respect to those in the dark was examined. Higher phosphatidate phosphatase-1 (PAP1) and 1-acylglycerol-3-phosphate O-acyltransferase (AGPAT) activities were found in retinal ganglion cells from animals exposed to light as compared to those maintained in the dark. By contrast, no dark/light differences were found in PSS 1 or 2 activities. Treatment of retinas with c-Fos ASO abrogated the activation of these enzyme activities [28].

In the pathway of glycolipid synthesis, the activities of glucosylceramide (GlcCer) galactosyltransferase 1 and lactosylceramide sialyltransferase 1 are essentially unaffected by c-Fos whereas c-Fos specifically activates the enzyme GlcCer synthase, the product of which, GlcCer, is the first glycosylated intermediate in the pathway of synthesis of glycolipids.

This activation leads to a global increment in ganglioside labeling. As occurred with enzymes of the pathway of phospholipid synthesis, c-Fos doubled the V_{max} of GlcCer synthase with no effect on its K_m [24,33].

Just as c-Fos has been found to be associated with the ER, so has been the activity of the enzymes it activates, which are totally or partially associated with this organelle. In this regard, it seems that there is some controversy concerning the localization of PI4KIIα; however, there has been a report on a minor but highly active pool of the enzyme in the ER [39]. Also, GlcCer synthase, which is typically classified as a Golgi enzyme, has also been detected in pre-Golgi/ER membranes [40,41]. Furthermore, GlcCerS is unique among glycolipid glycosyltransferases in the sense that its catalytic site is oriented toward the cytosolic face rather than to the luminal face of the ER [40]. As no activation by c-Fos of any enzyme outside the ER has been found, we conclude that the general lipid synthesis activation by c-Fos is achieved by increasing the activity of particular key enzymes that translocate to or are integral components of the ER.

Fig. 2 summarizes both activities of c-Fos, that is, as an AP-1 transcription factor that regulates the gene expression or as an activator of particular enzymes of the pathway of synthesis of PPIs. It should be noted that although it only schematizes the latter pathway, c-Fos increases the synthesis of the major phospholipids.

8. Molecular mechanism of c-Fos-dependent lipid synthesis activation

The ultimate experimental evidence for establishing the AP-1 independent mechanism for c-Fos-dependent activation of lipid synthesis came from the finding of another common feature of the phenomena: the activated enzymes physically interact with c-Fos. c-Fos co-immunoprecipitates only with the enzymes of the pathway of PPIs synthesis or of glycolipid synthesis whose activity it modulates but not with those it does not regulate. Furthermore, FRET microscopy (Förster Resonance Energy Transfer) ascertained a direct association between c-Fos and the enzymes CDS and PI4KIIα that it activates (Fig. 3) [33].

The c-Fos domain involved in its association with the enzymes it activates is its N-terminal domain: the mutants c-Fos:1–139 (aa 1–139), c-Fos:1–160 (aa 1–160), and ΔBD physically associate to CDS [28] and to PI4KIIα (unpublished), the activated enzymes of pathway of

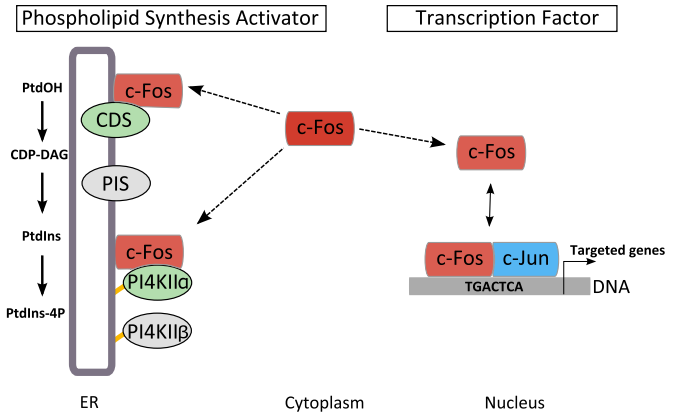

Fig. 2. Dual activities of c-Fos: as an activator of glycerolipid biosynthesis and as an AP-1 transcription factor. Left: c-Fos acts as a phospholipid synthesis activator by physically interacting with specific enzymes at the endoplasmic reticulum (ER) membrane. Enzymes in green are activated by c-Fos whereas gray colored ones are not. While CDS and PIS are constitutive residents of the ER membrane, one or more palmitoyl groups covalently linked tether PI4KII isoforms to the bilayer. Right: c-Fos's canonic function as an AP-1 transcription factor. c-Fos-containing AP-1 dimers enters the nucleus and recognizes target DNA sequences by means of a bipartite basic, DNA-binding domain.

synthesis of the PPIs. By contrast, the mutant c-Fos 165–380 that contains the leucine zipper (LZ) domain (aa 165–193) required for AP-1 formation up to the C-terminus of c-Fos does not associate with CDS. Interestingly, this c-Fos/enzyme association is promoted irrespective of the presence of the BD domain that is required to activate lipid synthesis as evidenced by c-Fos:1–139 and ΔBD that have no lipid synthesis activating capacity but associate with CDS and to PI4KIIα [33].

The simplest interpretation 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 any case, it is worth mentioning that no other known protein has been shown to be capable of binding to key phospholipid synthesis enzymes and of activating their catalytic capacity.

9. Future directions

The regulation of membrane biogenesis for the different stages of cell growth and proliferation is a key process that must be finely tuned because it involves the synchronous activity of several metabolic pathways orchestrated at multiple levels. By no doubt, a key mechanism to achieve global regulation of membrane biogenesis is through transcription factors that act coordinately to regulate the expression of

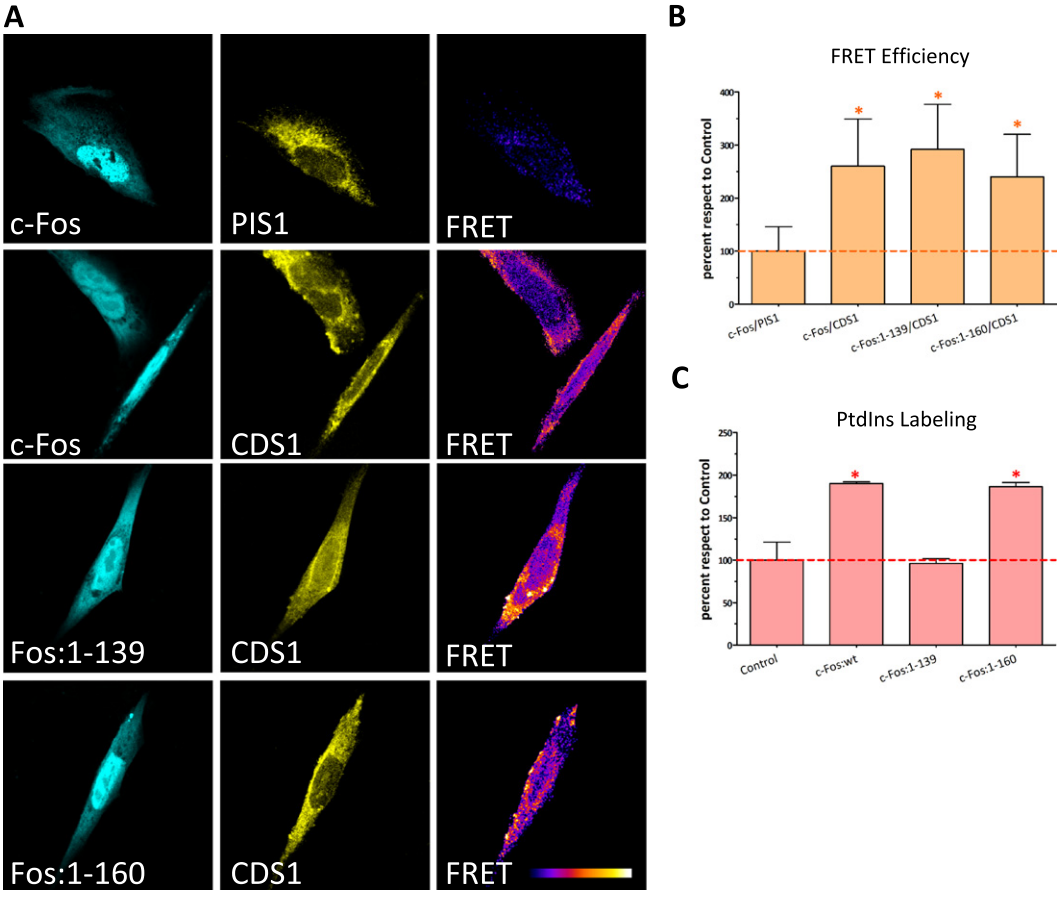

Fig. 3. c-Fos associates with the enzymes by its N-terminus whereas activation is accomplished through its BD domain. A. Cells co-transfected to express CFP-tagged c-Fos or c-Fos deletion mutants and the corresponding YFP-tagged enzyme (PIS1 or CDS1) were examined by confocal microscopy using filters for CFP (left) or for YFP (center). FRET efficiency images were obtained and pseudo-colored (right) using PFRET software. FRET bar shown on the right corresponds to a blue-to-yellow increasing scale of FRET efficiency. B. Mean FRET efficiencies ± SD for the donor/acceptor pairs shown in A. Note that the N-terminal domain of c-Fos (Fos:1–139) is sufficient to associate with the enzyme CDS1. *P < 0.001 C. PtdInsP labeling was determined in vitro in the presence of c-Fos or of its deletion mutants c-Fos:1–139 or c-Fos:1–160, at a final concentration of 1 ng/μg cell homogenate protein. *P < 0.01. Note that c-Fos deletion mutants containing its N-terminal domain plus its BD domain are sufficient to activate the labeling to levels comparable to those of full length c-Fos. Results from [33].

lipid synthesizing enzymes such as those that occur during cell differentiation [42]. As lipid biosynthesis involves multiple, complex enzymatic steps, the initial concept was that the regulation of lipid synthesis would necessarily require complex and varied regulatory mechanisms. Still, lipid synthesis regulation and consequently membrane biogenesis regulation may result much simpler than envisaged years ago in the light of the results from many laboratories showing that by regulating only key rate-limiting steps the entire pathway is regulated.

In this regard, the evidence obtained up to date indicates that by increasing the catalytic capacity of rate-limiting enzymes, c-Fos is increasing the availability of major lipids as found in all the experimental paradigms used so far [20,22,23,25]. They further point to a possible shared molecular mechanism to activate the different enzymes c-Fos regulates.

We are currently extending our initial studies to other lipid synthesis pathways, mainly the Kennedy pathway, to determine if c-Fos also regulates its key enzymes. In fact, unpublished results show that this seems to be the case. Further studies are required to precisely establish the enzymes that c-Fos activates and if in all cases the molecular mechanism is shared. Perhaps these studies will teach us how to limit the unrestricted proliferation and growth of tumor cells by interfering with c-Fos activated lipid synthesis.

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References

[1] G. van Meer, D.R. Voelker, G.W. Feigenson, Membrane lipids: where they are and how they behave, Nat. Rev. Mol. Cell Biol. 9 (2008) 112–124.
[2] M. Sud, E. Fahy, D. Cotter, A. Brown, E.A. Dennis, C.K. Glass, A.H. Merrill Jr., R.C. Murphy, C.R. Raetz, D.W. Russell, S. Subramaniam, Lmsd: lipid maps structure database, Nucleic Acids Res. 35 (2007) D527–D532.
[3] S. Lev, Non-vesicular lipid transport by lipid-transfer proteins and beyond, Nat. Rev. Mol. Cell Biol. 11 (2010) 739–750.
[4] O. Kuge, K. Hasegawa, K. Saito, M. Nishijima, Control of phosphatidylserine biosynthesis through phosphatidylserine-mediated inhibition of phosphatidylserine synthase I in Chinese hamster ovary cells, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 4199–4203.
[5] G.S. Attard, R.H. Templer, W.S. Smith, A.N. Hunt, S. Jackowski, Modulation of CTP: phosphocholine cytidylyltransferase by membrane curvature elastic stress, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 9032–9036.
[6] M.N. Ng, T.E. Kitos, R.B. Cornell, Contribution of lipid second messengers to the regulation of phosphatidylcholine synthesis during cell cycle re-entry, Biochim. Biophys. Acta 1686 (2004) 85–99.
[7] D. Shan, J.L. Li, L. Wu, D. Li, J. Hurov, J.F. Tobin, R.E. Gimeno, J. Cao, GPAT3 and GPAT4 are regulated by insulin-stimulated phosphorylation and play distinct roles in adipogenesis, J. Lipid Res. 51 (2010) 1971–1981.
[8] T.E. Harris, T.A. Huffman, A. Chi, J. Shabanowitz, D.F. Hunt, A. Kumar, J.C. Lawrence Jr., Insulin controls subcellular localization and multisite phosphorylation of the phosphatidic acid phosphatase, lipin 1, J. Biol. Chem. 282 (2007) 277–286.
[9] T. Curran, J.I. Morgan, Memories of fos, Bioessays 7 (1987) 255–258.
[10] B.L. Caputto, M.E. Guido, Immediate early gene expression within the visual system: light and circadian regulation in the retina and the suprachiasmatic nucleus, Neurochem. Res. 25 (2000) 153–162.
[11] P. Hughes, M. Dragunow, Induction of immediate-early genes and the control of neurotransmitter-regulated gene expression within the nervous system, Pharmacol. Rev. 47 (1995) 133–178.
[12] K. Kovary, R. Bravo, Existence of different fos/jun complexes during the g0-to-g1 transition and during exponential growth in mouse fibroblasts: differential role of fos proteins, Mol. Cell. Biol. 12 (1992) 5015–5023.
[13] S.P. Hunt, A. Pini, G. Evan, Induction of c-fos-like protein in spinal cord neurons following sensory stimulation, Nature 328 (1987) 632–634.
[14] P. Angel, I. Baumann, B. Stein, H. Delius, H.J. Rahmsdorf, P. Herrlich, 12-o-tetradecanoyl-phorbol-13-acetate induction of the human collagenase gene is mediated by an inducible enhancer element located in the 5′-flanking region, Mol. Cell. Biol. 7 (1987) 2256–2266.
[15] L.D. Kerr, D.B. Miller, L.M. Matrisian, TGF-beta 1 inhibition of transin/stromelysin gene expression is mediated through a fos binding sequence, Cell 61 (1990) 267–278.
[16] W. Lee, P. Mitchell, R. Tjian, Purified transcription factor AP-1 interacts with tpa-inducible enhancer elements, Cell 49 (1987) 741–752.
[17] J. Basbous, I. Jariel-Encontre, T. Gomard, G. Bossis, M. Piechaczyk, Ubiquitin-independent- versus ubiquitin-dependent proteasomal degradation of the c-fos and fra-1 transcription factors: is there a unique answer? Biochimie 90 (2008) 296–305.
[18] P. Tompa, C. Szasz, L. Buday, Structural disorder throws new light on moonlighting, Trends Biochem. Sci. 30 (2005) 484–489.
[19] A.M. Cardozo Gizzi, B.L. Caputto, Mechanistic insights into the nongenomic regulation of phospholipid synthesizing enzymes, IUBMB Life 65 (2013) 584–592.
[20] M.E. Guido, G.A. de Arriba Zerpa, D.F. Bussolino, B.L. Caputto, Immediate early gene c-fos regulates the synthesis of phospholipids but not of gangliosides, J. Neurosci. Res. 43 (1996) 93–98.
[21] D.F. Bussolino, G.A. de Arriba Zerpa, V.R. Grabois, C.B. Conde, M.E. Guido, B.L. Caputto, Light affects c-fos expression and phospholipid synthesis in both retinal ganglion cells and photoreceptor cells in an opposite way for each cell type, Brain Res. Mol. Brain Res. 58 (1998) 10–15.
[22] D.F. Bussolino, M.E. Guido, G.A. Gil, G.A. Borioli, M.L. Renner, V.R. Grabois, C.B. Conde, B.L. Caputto, C-fos associates with the endoplasmic reticulum and activates phospholipid metabolism, FASEB J. 15 (2001) 556–558.
[23] G.A. Gil, D.F. Bussolino, M.M. Portal, A. Alfonso Pecchio, M.L. Renner, G.A. Borioli, M.E. Guido, B.L. Caputto, C-fos activated phospholipid synthesis is required for neurite elongation in differentiating PC12 cells, Mol. Biol. Cell 15 (2004) 1881–1894.
[24] P.M. Crespo, D.C. Silvestre, G.A. Gil, H.J. Maccioni, J.L. Daniotti, B.L. Caputto, C-fos activates glucosylceramide synthase and glycolipid synthesis in PC12 cells, J. Biol. Chem. 283 (2008) 31163–31171.
[25] D.C. Silvestre, G.A. Gil, N. Tomasini, D.F. Bussolino, B.L. Caputto, Growth of peripheral and central nervous system tumors is supported by cytoplasmic c-fos in humans and mice, PLoS One 5 (2010) e9544.
[26] G.A. Gil, D.C. Silvestre, N. Tomasini, D.F. Bussolino, B.L. Caputto, Controlling cytoplasmic c-fos controls tumor growth in the peripheral and central nervous system, Neurochem. Res. 37 (2012) 1364–1371.
[27] R.D. Motrich, G.M. Castro, B.L. Caputto, Old players with a newly defined function: fra-1 and c-fos support growth of human malignant breast tumors by activating membrane biogenesis at the cytoplasm, PLoS One 8 (2013) e53211.
[28] G.A. de Arriba Zerpa, M.E. Guido, D.F. Bussolino, S.J. Pasquare, P.I. Castagnet, N.M. Giusto, B.L. Caputto, Light exposure activates retina ganglion cell lysophosphatidic acid acyl transferase and phosphatidic acid phosphatase by a c-fos-dependent mechanism, J. Neurochem. 73 (1999) 1228–1235.
[29] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (2011) 646–674.
[30] W. Cammer, F.A. Tansey, C.F. Brosnan, Gliosis in the spinal cords of rats with experimental allergic encephalomyelitis: immunostaining of carbonic anhydrase and vimentin in reactive astrocytes, Glia 2 (1989) 223–230.
[31] P. Angel, M. Karin, The role of jun, fos and the AP-1 complex in cell-proliferation and transformation, Biochim. Biophys. Acta 1072 (1991) 129–157.
[32] M.M. Portal, G.O. Ferrero, B.L. Caputto, N-terminal c-fos tyrosine phosphorylation regulates c-fos/er association and c-fos-dependent phospholipid synthesis activation, Oncogene 26 (2007) 3551–3558.
[33] A.R. Alfonso Pecchio, A.M. Cardozo Gizzi, M.L. Renner, M. Molina-Calavita, B.L. Caputto, C-fos activates and physically interacts with specific enzymes of the pathway of synthesis of polyphosphoinositides, Mol. Biol. Cell 22 (2011) 4716–4725.
[34] T. Sasaki, H. Kojima, R. Kishimoto, A. Ikeda, H. Kunimoto, K. Nakajima, Spatiotemporal regulation of c-Fos by ERK5 and the E3 ubiquitin ligase UBR1, and its biological role, Mol. Cell 24 (2006) 63–75.
[35] L.O. Murphy, J. Blenis, MAPK signal specificity: the right place at the right time, Trends Biochem. Sci. 31 (2006) 268–275.
[36] L.O. Murphy, S. Smith, R.H. Chen, D.C. Fingar, J. Blenis, Molecular interpretation of ERK signal duration by immediate early gene products, Nat. Cell Biol. 4 (2002) 556–564.
[37] G.O. Ferrero, F.N. Velazquez, B.L. Caputto, The kinase c-src and the phosphatase TC45 coordinately regulate c-fos tyrosine phosphorylation and c-fos phospholipid synthesis activation capacity, Oncogene 31 (2012) 3381–3391.
[38] T. Hunter, Tyrosine phosphorylation: thirty years and counting, Curr. Opin. Cell Biol. 21 (2009) 140–146.
[39] M.G. Waugh, S. Minogue, J.S. Anderson, A. Balinger, D. Blumenkrantz, D.P. Calnan, R. Cramer, J.J. Hsuan, Localization of a highly active pool of type ii phosphatidylinositol 4-kinase in a p97/valosin-containing-protein-rich fraction of the endoplasmic reticulum, Biochem. J. 373 (2003) 57–63.
[40] A.H. Futerman, R.E. Pagano, Determination of the intracellular sites and topology of glucosylceramide synthesis in rat liver, Biochem. J. 280 (Pt 2) (1991) 295–302.
[41] A. Kohyama-Koganeya, T. Sasamura, E. Oshima, E. Suzuki, S. Nishihara, R. Ueda, Y. Hirabayashi, Drosophila glucosylceramide synthase: a negative regulator of cell death mediated by proapoptotic factors, J. Biol. Chem. 279 (2004) 35995–36002.
[42] A. Nohturfft, S.C. Zhang, Coordination of lipid metabolism in membrane biogenesis, Annu. Rev. Cell Dev. Biol. 25 (2009) 539–566.