# Docosahexaenoic acid: membrane modification and neurotrophic mechanisms

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**Abstract:** *Docosahexaenoic acid (DHA, 22:6n-3) is highly enriched in the brain mainly in membrane phospholipids. Maintenance of a high DHA concentration in brain is essential for proper neuronal function, suggesting a unique requirement of this fatty acid for an optimal membrane structure and function in the nervous system. This article discusses mechanisms supporting neurotrophic function of DHA, particularly the role of DHA in membrane modification and related cell signaling leading to neuronal survival and differentiation.*

**Key words:** *docosahexaenoic acid, neuronal cells, phosphatidylserine, apoptosis, survival, neurite growth, synaptamide, synaptogenesis, synaptic function, Akt, Raf-1, membrane*

## DHA and membrane modification

DHA is highly concentrated in neuronal cells, particularly in aminophospholipids such as phosphatidylethanolamine (PE) and phosphatidylserine (PS) (Salem *et al.*, 2001). PS represents the major acidic phospholipid in eukaryotic cell membranes and participates in important signaling processes (Kim, 2007; Kim *et al.*, 2010). In animal cells, PS is synthesized from PC or PE by the serine base exchange reaction catalyzed by PS synthases (PSS) (Vance, 2008). The high compositional profile of DHA in PS can be attributed to the molecular species specificity in the PS synthesis or degradation, although deacylation/reacylation reactions may also contribute (Lands, 1960). The PS decarboxylation (PSD) occurs most preferably for 18:0, 22:6-PS (Kevala and Kim, 2001), suggesting that unfavored PS degradation of DHA-containing PS species is an unlikely cause for the high level of DHA species in PS. We have also demonstrated that 18:0,22:6-PC or PE is the best substrate for PSS1 (Kim *et al.*, 2004) or PSS2 (Wen and Kim, 2007), respectively, which contributes to the
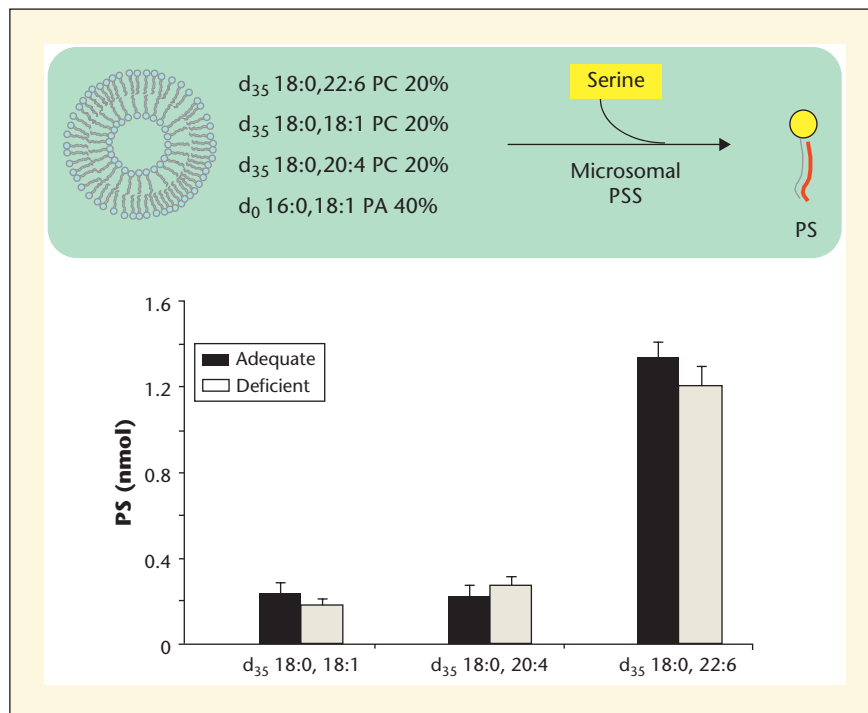


Figure 1. *Effects of n-3 fatty acid depletion on microsomal PS biosynthetic activity. Microsomes (750 μg protein) obtained from the rats raised with n-3 fatty acid sufficient or deficient diets were incubated with exogenously added deuterium labeled PC substrates (50 μM each) in the presence of 1.5 mM serine for 1 h. Deuterium labeled PS products were measured by liquid chromatography/electrospray ionization-mass spectrometry. Neither PS biosynthetic capacity nor substrate specificity was significantly altered due to the replacement of DHA with DPAn-6. (Z. Wen and H.Y. Kim, unpublished data).*
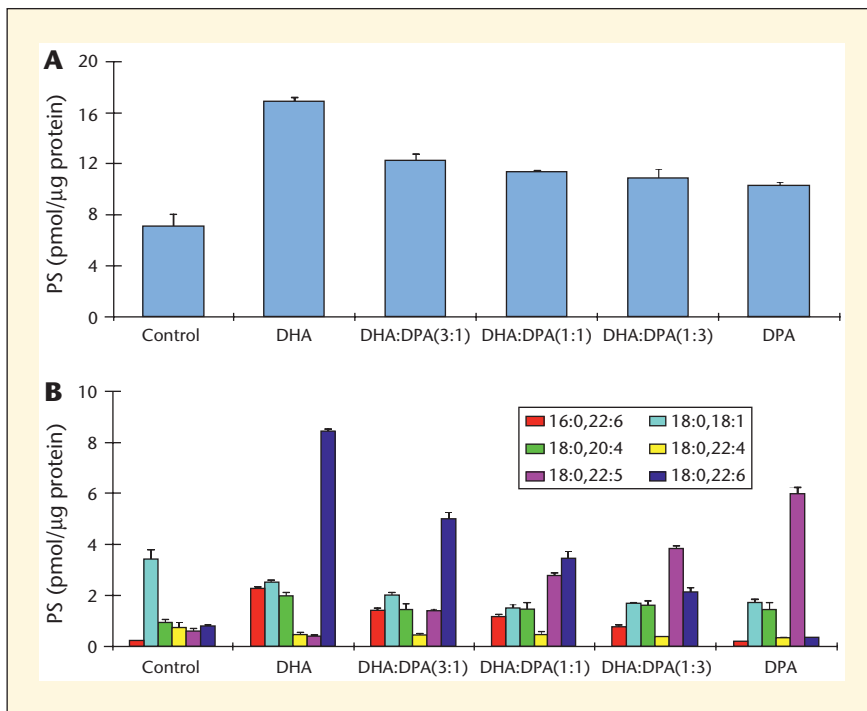
Figure 2. *Differential effects of DHA and DPAn-6 on PS accumulation. Neuro 2A cells were supplemented for 48 h with DHA and/or DPAn-6 at the final total fatty acid concentration of 25 μM, in the presence of 40 μM Vit E and 1% FBS. The total PS (A) and individual PS molecular species levels (B) were determined by liquid chromatography/electrospray ionization-mass spectrometry. (Z. Wen, M. Akbar and H.Y. Kim, unpublished data).*

concentration of DHA in PS. In neural tissues where DHA is highly concentrated, PS levels are also high, suggesting that DHA is a positive modulator for PS synthesis (Kim, 2008). Indeed, DHA increases the PS content in neuronal cells primarily due to the increased PS biosynthesis and accumulation of 18:0,22:6-PS (Garcia *et al.*, 1998). Among many cells in culture, the DHA-induced PS increase occurs uniquely in neuronal cells (Guo *et al.*, 2007). In contrast, depletion of DHA has a profound effect on the PS accumulation specifically in neural tissues where DHA is highly enriched (Kim, 2008; Hamilton *et al.*, 2000). N-3 fatty acid deficiency results in depletion of DHA, and thus PC and PE species containing DHA, the most preferred substrates for PS synthesis. The compensatory increase of docosapentaenoic acid (DPAn-6, 22:5n-6) is not sufficient to fully recover the PS level (Hamilton *et al.*, 2000), since DPAn-6-containing phospholipids are less effective substrates for PS synthesis in comparison to DHA-containing phospholipids (Kim *et al.*, 2004; Wen and Kim, 2007). N-3 fatty acid deficiency does not significantly affect microsomal

PSS activity itself *(figure 1)*, indicating that the observed PS decrease after n-3 fatty acid depletion is primarily due to the limited availability of DHA-containing phospholipids, the best substrates for PSS. In neuronal cells in culture, both DHA and DPAn-6 supplementation significantly increase the PS content *(figure 2A)*. However, DPAn-6-induced PS increase is less than 80% of the PS increase after DHA enrichment. The individual phospholipid molecular species distribution shows that 18:0, 22:6-PS increases more than 18:0, 22:5n-6 species after enrichment with DHA and DPAn-6 at an equal concentration *(figure 2B)*. As the proportion of DHA with respect to DPAn-6 decreases, a gradual reduction in the total PS content is observed. Findings from both *in vivo* n-3 fatty acid depletion and in cell supplementation with DHA or DPAn-6 consistently indicate that neuronal PS accumulation is dependent on the DHA content. Indeed, DHA enrichment is a unique mechanism to alter the PS pool, which is limited to neuronal membranes. The plasma membrane and recycling endosomes are the major sites where PS localizes in the cytoplasmic face

(Calderon and Kim, 2008), suggesting that these membranes can offer specific target surface for cytosolic signaling proteins that interact with PS.

## DHA and neuronal survival

The biochemical characteristics of DHA to increase PS have significant implication in neuronal survival. Supplementation of neuronal cells with DHA reduces caspase-3 activation, a hallmark for apoptotic cell death, induced by serum deprivation *(figure 3)*. This protection occurs only when the PS pool is allowed to expand as the supplementation with oleic acid (18:1n-9, OA) or DHA under a serine-free condition is not effective, indicating that DHA prevents apoptotic cell death in a PS-dependent manner (Kim *et al.*, 2000). Adverse effects of n-3 fatty acid deficiency on cell survival is readily apparent due to the differential capacity of DHA and DPAn-6 to accumulate PS in neuronal membranes following *in vivo* dietary manipulation of n-3 fatty acid intake or supplementation of neuronal cells in culture (Akbar *et al.*, 2005). Neuronal apoptosis induced by staurosporine (ST) treatment is another apoptotic model where DHA inhibited cell death due to its ability to promote PS accumulation in cell membranes (Akbar and Kim, 2002). These findings indicate a unique role of DHA in neuronal survival through modulating membrane PS levels which in turn can influence PS-dependent signaling events.

## DHA and neurodevelopment

The importance of developmental accretion of DHA in hippocampus-dependent function such as learning and memory in humans and animals has been well documented (Willatts *et al.*, 1998; Birch *et al.*, 2000; Gamoh *et al.*, 1999; Moriguchi *et al.*, 2000). Previously, we have demonstrated that DHA uniquely increases the population of neurons with longer neurites and a higher number of branches in rat and mouse embryonic hippocampal neuronal cultures (Moriguchi *et al.*, 2000; Cao *et al.*, 2009). Similar stimulating effects of DHA on neurite growth can be also observed in cortical neurons *(figure 4)*. It has been demonstrated in mouse hip-
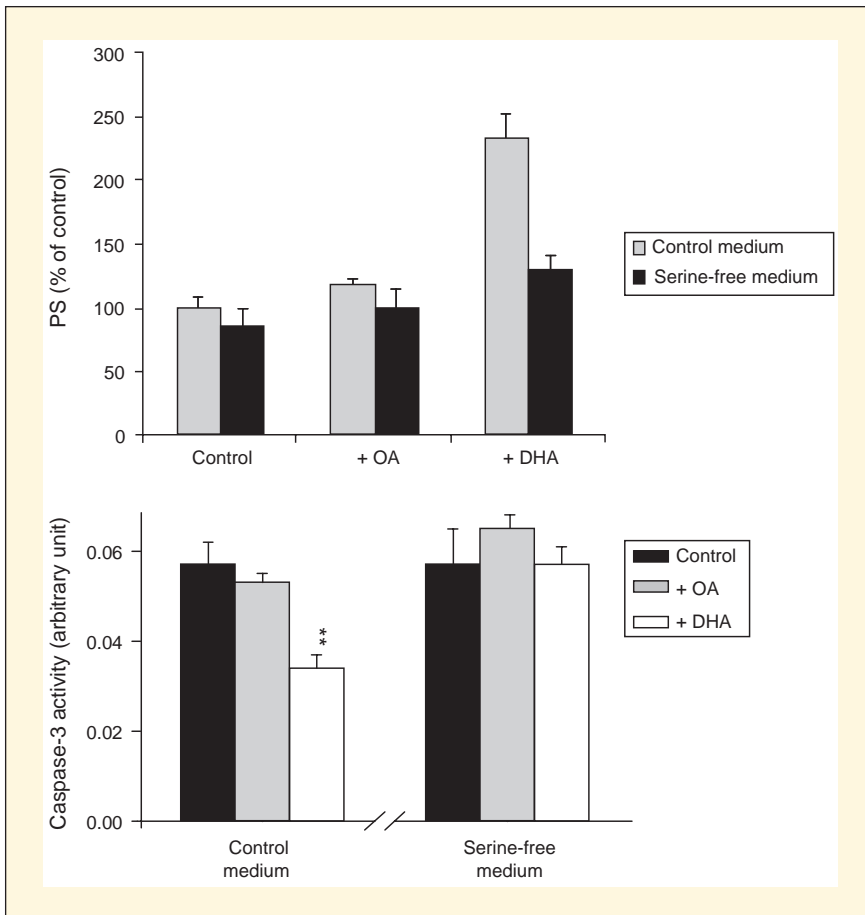
Figure 3. *Neuronal survival promoted by DHA in a PS-dependent manner (from Kim et al., 2000). Neuro 2A cells were supplemented with 25 μM DHA or oleic acid (OA) for 2 days in the control or serine-free media, and the PS content and caspase-3 activity induced by 24 h serum deprivation were evaluated.*

pocampal cultures that DHA promotes not only neurite growth but also synaptogenesis and the expression of pre- and post-synaptic proteins such as synapsins and N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazole (AMPA) receptors, enhancing glutamatergic synaptic activity (Cao *et al.*, 2009). Moreover, developmental depletion of DHA results in reduced expression of those synaptic proteins in the hippocampi of 18-day-old mice. Such reduction significantly impairs long-term potentiation (LTP), a well characterized form of synaptic plasticity similar to that involved in hippocampus-based learning and memory. Rat hippocampal neurons similarly increase neurite growth and synaptogenesis after DHA treatment *(figure 5)*. Recently, N-docosahexaenoylethanolamide (Synaptamide, DEA) has been identified as a bioactive DHA metabolite endogenously formed in the brain. This amide form of DHA metabolite is a potent mediator for DHA-induced neurite growth, synaptogenesis and glutamatergic synaptic function (Kim *et al.*, 2011), and thus named as synaptamide (Kim *et al.*, 2012). The formation of synaptamide as well as its unique signaling is expected to play a significant role in neuronal development. In addition, DHA-mediated modification of membrane PS content may also serve as an important mechanism for DHA-induced neuritogenesis, synaptogenesis and synaptic function, as in the case of neuronal survival.

## Membrane modification and cell signaling

The biochemical function of DHA to promote PS accumulation in the central nervous system is an important underpinning of the maintenance of neuronal survival, since the protection by DHA is PS-dependent. Specifically, facilitated PS-dependent Akt translocation is a target event where the protective effect of DHA on neuronal survival is derived (Akbar, *et al.*, 2005). It is well established that Akt activation requires phosphatidylinositol 3,4,5-trisphosphate ($PIP_3$)-dependent membrane translocation and phosphorylation (Alessi and Cohen, 1998). Growth factor receptor stimulation activates PI3 kinase (PI3K), producing $PIP_3$ which in turn recruits cytosolic Akt to the plasma membrane through the interaction with the pleckstrin homology domain (PH) of Akt. We have recently demonstrated a novel molecular mechanism revealing that Akt activation is critically dependent on the interaction with not only well-recognized $PIP_3$ but also PS (Huang *et al.*, 2011). In addition, we have provided a molecular basis for the DHA-promoted neuronal survival by
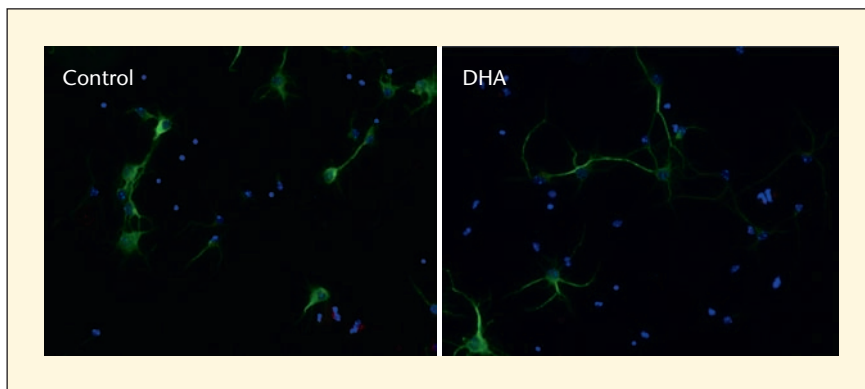


Figure 4. *Neurite growth stimulated by DHA in embryonic cortical neurons. E18 cortical neurons were cultured with or without 1 μM DHA for 3 days. Neurite development was visualized by MAP2 immunostaining. (J.W. Lee, G. Kharebava and H.Y. Kim, unpublished data).*
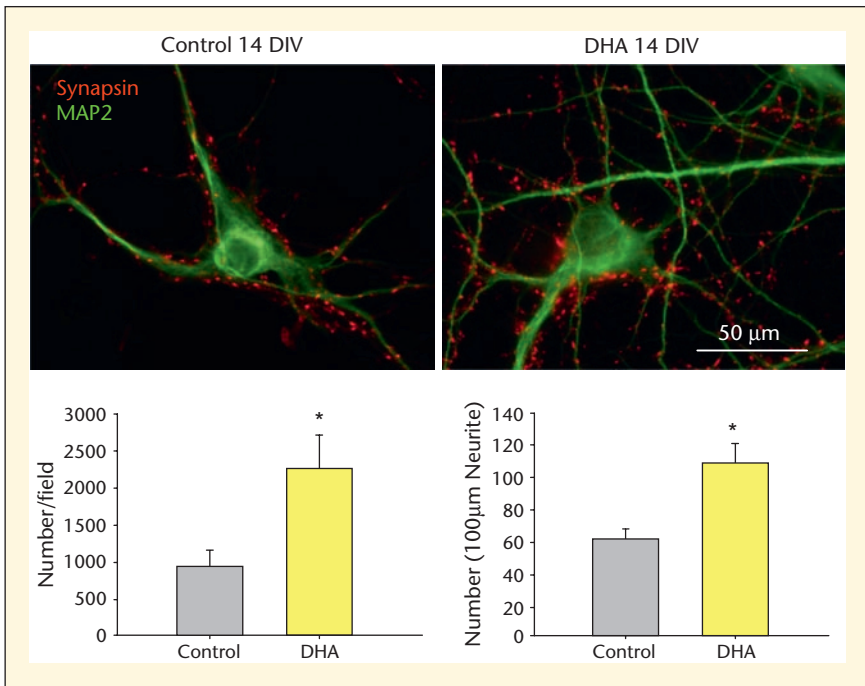
Figure 5. *Synaptogenesis promoted by DHA in rat embryonic hippocampal neuronal cultures. E18 rat hippocampal neurons were cultured in the presence or absence of 2 µM DHA for 14 days (14DIV), and neurite growth and synapsin puncta formation were visualized by MAP2 and synapsin immunostaining, respectively. (F. Calderon and H.Y. Kim, unpublished data).*

demonstrating that Akt translocation and activation is affected by Akt-PS interaction in a PS concentration-dependent manner. The decreased PS content in neural membranes due to DHA depletion (Hamilton *et al.*, 2000) would diminish Akt activation and thus increase neuronal cell death, particularly under adverse conditions where PIP$_3$ is limited (Akbar *et al.*, 2005; Akbar and Kim, 2002). In contrast, increasing the plasma membrane PS content by DHA supplementation would facilitate cellu-

lar Akt activation, improving neuronal survival. Considering the necessity of PS-Akt interaction for Akt activation, DHA's capacity to increase PS is of crucial importance to sustain survival of neurons particularly under distress *(figure 5)*.

Raf-1 activation, which is an upstream event of MAP kinase activation, is known to play an important role in transducing signals of many growth factors, and thus influencing cell survival, differentiation and proliferation

(Kim *et al.*, 2010). The importance of Raf-1 activation in neurite growth is evident as Neuro 2A cells expressing a constitutively active Raf-1 mutant (Raf-1K375M) shows considerably improved neurite outgrowth in comparison to the cells expressing wild type Raf-1 *(figure 6)*. Although the mechanism of Raf-1 activation is complex, translocation of Raf-1 to the membrane and subsequent phosphorylation are considered to be important steps for its activation (Stokoe *et al.*, 1994). It has been shown that Raf-1 kinase contains distinct binding domains for acidic phospholipids (Improta-Brears *et al.*, 1999), and therefore, membrane localization of Raf-1 may be dependent on the concentration of PS modulated by DHA. According to our previous finding, membrane translocation of Raf-1 in response to BDNF is indeed significantly enhanced by DHA enrichment in Neuro 2A cells (Kim *et al.*, 2000). Therefore, it is conceivable that Raf-1 activation facilitated by DHA-induced PS increase in the plasma membrane at least in part contributes to the neuritogenesis and synaptogenesis mediated by DHA.

## Conclusion

One of the specific biochemical functions of DHA in the central nervous system is to increase the PS content primarily through the accumulation of the molecular species 18:0,22:6-PS. Preferred microsomal PS synthesis from 18:0,22:6-PC appears to be a mechanism for the enrichment of this PS species. Depletion of DHA by n-3 fatty acid deficiency has a significant negative impact on the PS accumulation in neuronal cells due to the fact that DPAn-6, the substitute for DHA under this condition, does not fully support the original level of PS biosynthesis. DHA supports neuronal survival in a PS-dependent manner, particularly under adverse conditions. The PS-dependent acceleration of Akt translocation is particularly vital under suboptimal conditions where the survival signal is compromised. DHA also promotes neurite growth, synaptogenesis and expression of synaptic proteins, improving synaptic function, at least partly through facilitating Raf-1 activation. *Figure 7* summarizes membrane PS-related signaling mechanisms invloved in DHA-mediated neuroprotection. Taken together, one of the primary biological
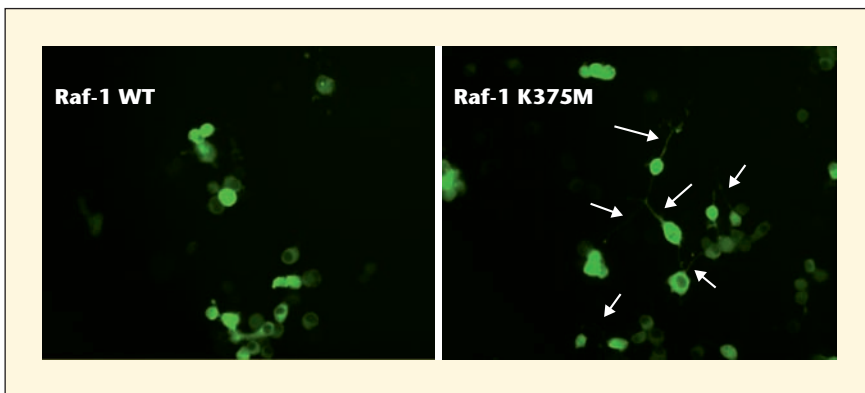


Figure 6. *Neurite growth promoted by active Raf-1. Neuro 2A cells were transfected with wild type GFP-Raf-1 (WT) or constitutively active GFP-Raf-1 mutant (K375M) and neurite growth was subsequently observed. (F. Calderon and H.Y. Kim, unpublished data).*
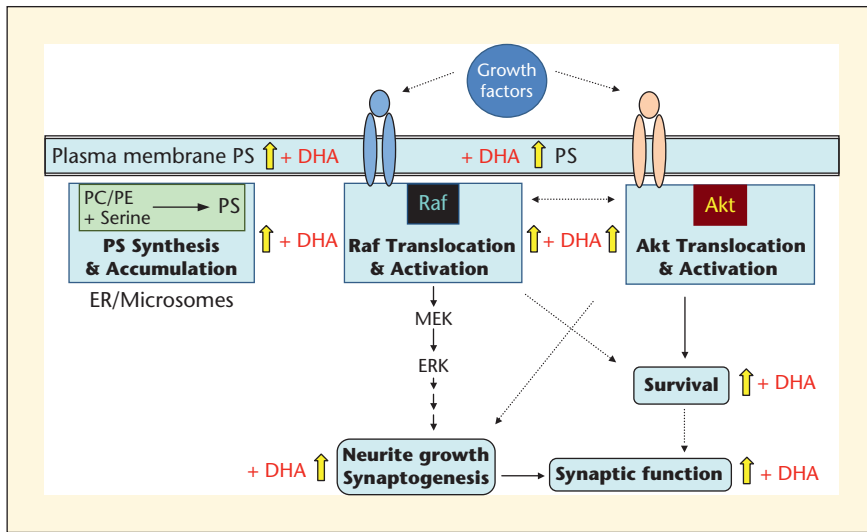
Figure 7. *Membrane PS-related signaling involved in DHA-mediated neuronal survival, neurito-genesis and synaptogenesis.*

functions of DHA is the maintenance of PS accumulation in the central nervous system due to its ability to positively modulate PS biosynthesis. In this regard, the loss of PS by n-3 fatty acid depletion and the resulting increased susceptibility to cell death and inadequate cellular development may have significant implications in neuronal dysfunction.

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