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Supernatant protein factor and tocopherol-associated protein: an unexpected link between cholesterol synthesis and vitamin E (Review)

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Abstract

Supernatant protein factor (SPF) is a recently cloned member of a family of cytosolic lipid-binding proteins that includes Sec14p, α-tocopherol transfer protein, and cellular retinal-binding protein. SPF stimulates the conversion of squalene to lanosterol in the downstream pathway for cholesterol biosynthesis, and overexpression of cloned SPF in hepatoma cells increases cholesterol synthesis. The mechanism of this stimulation has yet to be defined, but SPF appears to facilitate the transfer of squalene into and between intracellular membranes. The recent identification of SPF as α-tocopherol-associated protein (TAP) has called into question its long-standing association with cholesterol biosynthesis. TAP binds α-tocopherol, but not other isomers of tocopherol, with high affinity; in the presence of α-tocopherol TAP translocates to the nucleus and activates reporter gene transcription. Given the ability of α-tocopherol to down-regulate the expression of two scavenger lipoprotein receptors, SR-A and CD36, these observations raise some interesting questions regarding the role of SPF/TAP and vitamin E in cholesterol metabolism.

Keywords: Squalene monooxygenase; Oxidosqualene cyclase; Squalene; Lipoprotein receptors

1. Supernatant protein factor

Supernatant protein factor (SPF) was discovered in the early 1970's in Konrad Bloch's laboratory during the course of investigations on squalene monooxygenase, the first oxidative enzyme in cholesterol biosynthesis. Early studies with microsomal preparations of squalene monooxygenase required the addition of the 100,000 $\times$ g supernatant fraction to the incubations for activity [1,2], suggesting that one or more cytosolic factors was necessary for squalene monooxygenase activity. These cytosolic factors were subsequently shown to consist of FAD, an anionic phospholipid (phosphatidylserine, phosphatidylinositol, or phosphatidylglycerol), and a 47 kDa heat-labile protein termed "supernatant protein factor" [2,3]. Little activity was obtained if phospholipid or SPF was omitted from the reaction mixture, and partially purified SPF could not be replaced by bovine serum albumin (BSA) or high-density lipoprotein. Surprisingly, SPF could not be shown to bind squalene or 2,3-oxidosqualene, arguing against a substrate or product carrier role.

1.1. Membrane dependency of SPF

Ono and Bloch [4] noted that the stimulatory effect of SPF was lost when squalene monooxygenase was solubilized and partially purified, suggesting that SPF is effective only with membrane-bound enzyme. Subsequent studies [5] suggested that SPF facilitates the movement of squalene between microsomal membrane compartments, enhancing the access of this lipophilic substrate to the membrane-bound enzyme. SPF also activates oxidosqualene cyclase, the second enzyme in the pathway from squalene to lanosterol [6,7]. As oxidosqualene cyclase is thought to be associated with the luminal surface of the microsomal membrane and thus not in direct contact with cytosolic components (including SPF), these findings support the view that SPF facilitates the transfer of squalene and oxidosqualene between membrane compartments, rather than interacting directly with these enzymes. SPF does not activate enzymes downstream of oxidosqualene cyclase in

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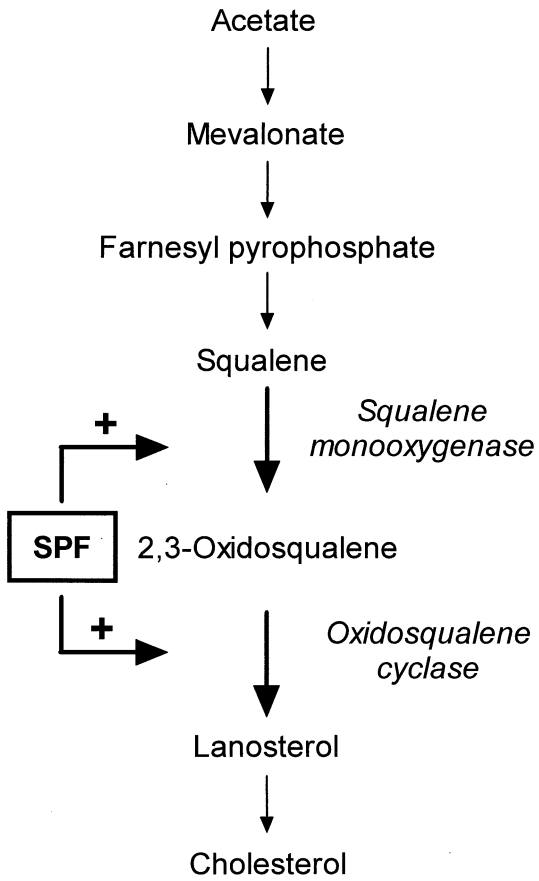

Fig. 1. Cholesterol synthesis and the sites of stimulation by SPF. Each arrow represents one or more enzymatic steps in cholesterol biosynthesis. SPF stimulates squalene monooxygenase and oxidosqualene cyclase.

the cholesterol synthesis pathway, indicating that it is specific for the squalene-to-lanosterol conversion steps (Fig. 1).

1.2. SPF promotes squalene uptake and transfer

Although the preponderance of evidence indicates that SPF promotes the transfer of squalene between membrane compartments, the mechanism of this transfer remains unresolved. As noted above, SPF cannot be shown to bind squalene, 2,3-oxidosqualene, or lanosterol. Based on radiolabeling studies, SPF does not promote the fusion of membranes or the transfer of phospholipid between membrane vesicles [8], and SPF is distinct from the phospholipid transfer proteins PLTP and PITP [9,10]. In addition to facilitating squalene transfer between membranes, SPF, along with anionic phospholipid, must be present for efficient uptake of exogenous (dietary) squalene into microsomes. Notably, once the squalene is incorporated into the membrane SPF does not need to be present for maximal stimulation of squalene monooxygenase activity [11]. As dietary squalene reaches the liver bound to serum lipoproteins, SPF may play a role in the regulation of cholesterol synthesis from dietary squalene by controlling its uptake by the endoplasmic reticulum.

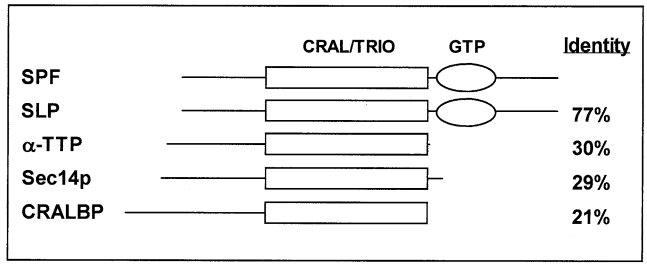

Fig. 2. Structural similarities between SPF and related proteins. Each protein contains a CRAL/TRIO domain, based the structure of Sec14p; SPF and SLP share a putative GTP-binding domain. Sequence identity of each protein with SPF is indicated on the right.

1.3. SPF is related to other lipid binding/transfer proteins

In the past year cDNAs for rat and human SPF were cloned by Shibata et al. [12]. Each encoded a 46 kDa, 403-amino acid protein. Comparison of the SPF sequences to the protein database revealed that SPF shows significant similarity to several proteins involved in lipid binding and transfer, including Sec14p, α-tocopherol transfer protein (α-TTP), and cellular retinal binding protein (CRALBP). The exact roles of these proteins are unclear; Sec14p is a yeast protein that appears to be involved in phosphatidylcholine/phosphatidylinositol exchange necessary for Golgi function; α-TTP is involved in regulating plasma vitamin E levels; and CRALBP binds 11-*cis*-retinal in mammalian visual organs. Although all of these proteins contain a 185-amino acid CRAL/TRIO domain thought to be involved in lipid binding, SPF contains a 157-amino acid carboxyl-terminal extension on this domain. SPF also shows 77% identity to a protein of unknown function isolated from rat olfactory epithelium [13]. This olfactory protein, termed SPF-like protein (SLP), had previously been shown to bind GTP, and, indeed, was identified and isolated on this basis; a GTP-binding motif is found at the beginning of the carboxyl-terminal extension that is common to SPF and SLP [14] (Fig. 2). Notably, SPF was earlier reported to be inhibited by nucleotides, including ATP and GTP; nucleotide binding was stoichiometric, although the affinity of SPF for various nucleotides was not reported [15].

1.4. SPF up-regulates cholesterol synthesis

Shibata et al. [12] showed that recombinant SPF exhibits many of the characteristics of native SPF: The cytosol from McARH7777 cells transfected with a rat SPF cDNA expression plasmid efficiently activated squalene monooxygenase in rat liver microsomes, and the purified protein catalyzed the transfer of radiolabeled squalene from liposomes to rat liver heavy membranes. This transfer was specific to SPF: α-TTP and BSA were inactive, and transfer could be reduced by unlabeled squalene and by 2,3-oxidosqualene, but not by farnesylpyrophosphate, lanosterol, or cholesterol. To address the role of SPF in cholesterol synthesis, lipids were

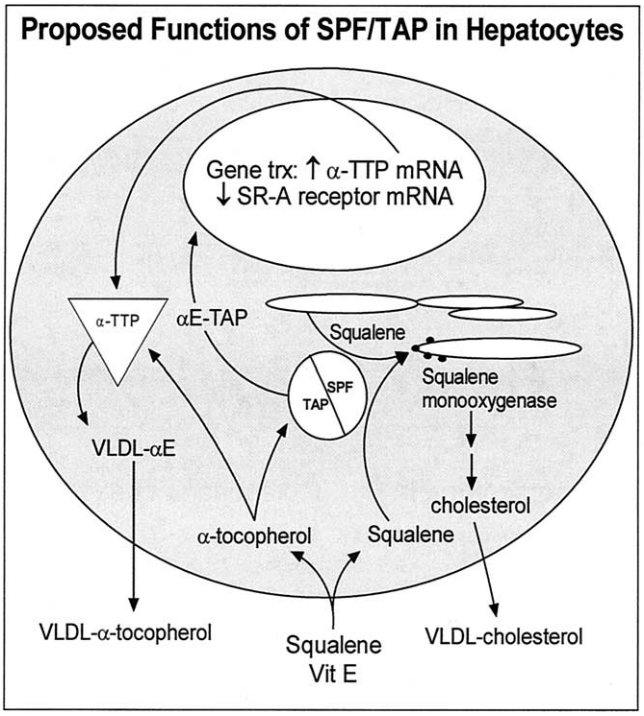

Fig. 3. Proposed functions of SPF/TAP in hepatocytes. Some of the possible roles of SPF/TAP in cholesterol synthesis and vitamin E processing are illustrated.

isolated from McARH7777 cells incubated with ^{14}C-acetate. SPF overexpression stimulated cholesterol synthesis by more than two-fold in these cells, and also increased the synthesis of squalene and lanosterol. As only an estimated 10% of the cells contained the SPF cDNA in this transient transfection assay, this represents a 20-fold increase in cholesterol biosynthesis in these cells. Moreover, the increase in squalene and lanosterol synthesis suggests a generalized up-regulation of the cholesterol biosynthetic pathway with SPF overexpression.

2. α-Tocopherol-associated protein, vitamin E, and atherosclerosis

The antioxidant effects of vitamin E are well known and are believed to play a role in the ability of this vitamin to reduce the incidence of coronary heart disease [16]. α-Tocopherol reduces low-density lipoprotein (LDL) oxidation [17] and prevents endothelial cell injury from oxidized lipids [18]. More recently, some of the actions of vitamin E have been suggested to be independent of its antioxidant effects. α-Tocopherol, but not β-tocopherol or other vitamin E isomers, inhibits protein kinase C and thereby decreases arterial smooth muscle cell proliferation [19]. Notably, α-tocopherol down-regulates the expression of the cholesterol scavenger receptors SR-A [20] and CD36 [21,22] via mechanisms that appear independent of protein kinase C and antioxidant activity. Regulation of these sterol receptors occurs at the level of transcription, suggesting that α-tocopherol acts through specific receptors or tocopherol-responsive transcription factors [23]. α-Tocopherol similarly upregulates the expression of α-TTP, and thus plays a role in its own intracellular processing [24,25]. These findings provide a link between vitamin E and the regulation of cholesterol synthesis that is independent of the antioxidant effects of vitamin E.

2.1. SPF is identical to α-tocopherol-associated protein (TAP)

α-Tocopherol-associated protein (TAP) is a recently identified cytosolic protein thought to be involved in the intracellular distribution of α-tocopherol [26]. Unexpectedly, the sequence of TAP is *identical* to that of SPF. TAP mRNA is expressed in most human tissues, with greatest expression in liver, brain, and prostate; SPF mRNA exhibits a similar expression pattern in rat, with greatest expression in liver, small intestine, brain, skin, and lung [12]. Although an exact function for TAP has not been determined, TAP binds to biotinylated α-tocopherol with a Kd of 0.46 μM; other forms of vitamin E, including β-, γ-, and δ-tocopherols, are bound with much lower affinity [26,27]. Very recent evidence suggests that TAP may act as an α-tocopherol-dependent transcription factor: A green fluorescent protein-TAP fusion protein expressed in COS-7 cells localizes in the cytoplasm in the absence of α-tocopherol, but translocates to the nucleus upon addition of 50 μM α-tocopherol [27]. Moreover, a GAL4-DNA-binding domain/TAP fusion protein activates GAL-dependent luciferase gene expression over 5-fold from a reporter plasmid in the presence of 50 μM α-tocopherol. Thus, TAP (SPF) may be involved in the α-tocopherol-dependent regulation of the scavenger cholesterol receptors noted above. It should be noted that this is not the only instance of a single protein with two very different functions: aconitase, an iron-sulfur protein that converts citrate to isocitrate in the tricarboxylic acid cycle, also serves as a translational regulator of proteins involved in cellular iron metabolism [28].

2.2. How can the very different actions of SPF and TAP be reconciled into one protein?

These results raise some compelling questions regarding the function of SPF/TAP. While it is evident that SPF enhances squalene monooxygenase activity *in vitro*, squalene binding cannot be demonstrated, and a mechanism for promoting squalene transfer into, between, and within membranes has eluded explanation. The possibility that SPF might act as a signaling molecule or transcription factor for cholesterol synthesis is intriguing, and is supported by the nucleotide inhibition studies [15] and the recent studies on the overexpression of SPF in hepatoma cells [12]. How vitamin E fits into this scheme is particularly puzzling; TAP evidently responds to α-tocopherol as a receptor/transcrip-

tion factor, and several of the genes affected by tocopherol levels are involved in cholesterol homeostasis. Understanding how these seemingly unrelated pathways for cholesterol synthesis and vitamin E signaling merge through this singular protein should be of considerable interest to researchers in the fields of lipid biochemistry and nutrition (Fig. 3).

Note added in proof:

The crystal structure of SPF/TAP was recently reported [29].

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