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Disentangling biological signaling networks by dynamic coupling of signaling lipids to modifying enzymes

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Abstract

An unresolved problem in biological signal transduction is how particular branches of highly interconnected signaling networks can be decoupled, allowing activation of specific circuits within complex signaling architectures. Although signaling dynamics and spatiotemporal mechanisms serve critical roles, it remains unclear if these are the only ways cells achieve specificity within networks. The transcription factor Steroidogenic Factor-1 (SF-1) is an excellent model to address this question, as it forms dynamic complexes with several chemically distinct lipid species (phosphatidylinositols, phosphatidylcholines and sphingolipids). This property is important since lipids bound to SF-1 are modified by lipid signaling enzymes (IPMK & PTEN), regulating SF-1 biological activity in gene expression. Thus, a particular SF-1/lipid complex can interface with a lipid signaling enzyme only if SF-1 has been loaded with a chemically compatible lipid substrate. This mechanism permits dynamic downstream responsiveness to constant upstream input, disentangling specific pathways from the full network. The potential of this paradigm to apply generally to nuclear lipid signaling is discussed, with particular attention given to the nuclear receptor superfamily of transcription factors and their phospholipid ligands.

Signaling Networks are highly interconnected

A major discovery in biological signaling over the past two decades has been that intracellular signal transduction occurs through highly integrated networks, rather than through isolated, linear signaling pathways (Fig 1) (Dutkowski et al., 2013; Guruharsha et al., 2012; Kapp et al., 2012; van Wageningen et al., 2010; Weng et al., 1999). These networks permit signaling crosstalk and other interactions that give rise to emergent network properties which potentially deliver far greater richness to biological signaling than had been realized (Bhalla and Iyengar, 1999; Helikar et al., 2008). Specifically, networks provide the cell with the capacity to store information within the network, outside the genome (Bhalla and Iyengar, 1999; Helikar et al., 2008), cluster standardized signaling output responses in the presence of high levels of background noise (Bhalla and Iyengar, 1999; Fritsche-Guenther et al., 2011; Helikar et al., 2008; Levy and Siegal, 2012), provide robustness to fate decisions and responsiveness to environmental change (Ku et al., 2012; Levy and Siegal, 2012), among other advantages (Bhalla et al., 2002). Given that signaling network crosstalk has been described in organisms from *E. coli* (Antiqueira et al., 2012) through metazoans (Natarajan et al., 2006) and humans (Ku et al., 2012), it seems clear that

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evolution has chosen complex, highly integrated networks as the preferred mode of intracellular information transfer (Kulkarni, 2013).

However, networks are not without at least one significant problem, which is also a great strength – the ability to cluster multiple signals into a few uniform outputs (Ku et al., 2012; Levy and Siegal, 2012). This property gives rise to the idea that "everything regulates everything else" and fuels the question of what mechanisms cells have evolved to selectively activate particular signaling molecules and pathways (Kiel and Serrano, 2012b), without inevitably having broad pleiotropic effects on the entire signaling architecture (Granek et al., 2011; Guruharsha et al., 2012; Kiel and Serrano, 2012a).

Known mechanisms of network disentanglement

There are several solutions to this problem that have already been characterized, and the field of signaling dynamics has realized some of the most elegant and effective answers to this basic problem. Altering the frequency or amplitude of signaling events can disentangle signaling networks in many ways (Ganesan and Zhang, 2012), however an excellent illustration is the Crz1 transcription factor (Cai et al., 2008). When the amplitude of Crz1 activation (the concentration of active Crz1) is steadily increased, Crz1 target promoters are non-proportionally activated according to the affinity of Crz1 for each promoter. However, when the frequency of Crz1 activation is increased instead of the amplitude, all Crz1 target promoters are activated proportionally, regardless of the affinity of Crz1 for each promoter (Cai et al., 2008). In other words, target promoter activation is non-proportional across Crz1 amplitudes, but proportional across Crz1 frequencies. In this way downstream effectors can be differentially regulated while still integrated into the network, simply by altering the amplitude or frequency of signaling (Purvis and Lahav, 2013). This highlights how dynamics can use an intrinsic network property to impart specificity and control within a complex system.

There are also less abstract, spatiotemporal mechanisms that confer specificity to signaling networks (Scott and Pawson, 2009; Steinacher and Soyer, 2012). These mechanisms generally rely on organizing signaling molecules in time and space to gain specificity by putting the right enzymes and substrates together at the right time. This can be accomplished through controlling enzyme/substrate concentration by post-translational modification, expression or degradation (Buchler and Cross, 2009; Rocks et al., 2005; Yu et al., 2011), sub-cellular localization and compartmentalization (Cohen and Cohen, 1989; Grecco et al., 2011; Kurosaki, 2002; Rocks et al., 2005), regulating the formation of multi-subunit complexes (Good et al., 2011; von Kriegsheim et al., 2009), and signal channeling through properly oriented and positioned signaling enzymes (Good et al., 2009; Rajakulendran et al., 2009; Whitmarsh et al., 1998). There are many examples of these well-characterized mechanisms throughout biology, which have been very well reviewed (Good et al., 2011; Kholodenko et al., 2010; Scott and Pawson, 2009).

Here, I attempt to present a new way cells can activate specific branches of complex networks that does not rely on time, space or signaling dynamics. This mechanism uses the chemical specificity encoded within lipid headgroups to interface between signaling enzymes and protein effectors. It is not exclusive of the above-mentioned strategies, and often may synergize with other mechanisms to enhance their effectiveness. The first example of this type of regulation centers on the phospholipid-regulated transcription factor Steroidogenic Factor-1.

Steroidogenic Factor-1 (SF-1) as a model system

Steroidogenic Factor-1 (SF-1, NR5A1) is a member of the nuclear receptor superfamily of ligand-regulated transcription factors, and is expressed in humans almost exclusively in tissues involved in steroid production (Morohashi et al., 1995). SF-1 binds specific DNA sequences in chromatin and recruits other transcriptional co-regulators to the promoters of genes (Lund et al., 2002). This action activates SF-1 target genes, which mainly encode steroidogenic enzymes (Omura and Morohashi, 1995). Many nuclear receptors (NRs) bind hydrophobic ligands that regulate their gene expression activity (Issemann and Green, 1990), and NRs are often regulated by several chemical variations on these hydrophobic molecules (Burris et al., 2013), making them attractive targets for pharmaceutical intervention.

SF-1 binds many different lipid species

Crystallographic structure analysis of SF-1 from several labs (Krylova et al., 2005; Li et al., 2005; Ortlund et al., 2005) unexpectedly demonstrated electron density attributed to a co-purifying bacterial phospholipid in the ligand-binding pocket of SF-1 (Fig. 2A) (Krylova et al., 2005). These studies also showed that SF-1 binds more physiologically relevant metazoan lipids *in vitro*, including important signaling lipids such as sphingosines, phosphatidylcholines and phosphatidylinositols (Dammer et al., 2007; Krylova et al., 2005; Li et al., 2005; Urs et al., 2007; Urs et al., 2006). The bacterial phospholipid structures were supported by an additional crystal structure of SF-1 bound to phosphatidylcholine (Fig. 2B) (Sablin et al., 2009), one of the most abundant phospholipids in eukaryotic nuclei (Hunt, 2006). Designed mutations in SF-1 that perturb phospholipid binding (Sablin et al., 2009; Sablin et al., 2003) also decrease SF-1 activation of every target gene that has been examined (Blind et al., 2012; Sablin et al., 2009), indicating that lipid binding is important for SF-1 biological activity. All current PDB crystal structures of SF-1 bound to various phospholipids indicate a similar mode of stoichiometric 1:1 binding, in which the hydrophobic acyl chains of the phospholipid are buried deep in the ligand-binding pocket of SF-1, while the hydrophilic headgroups are exposed to solvent (Fig 2C and 2D). The physiological reason phospholipids activate SF-1, a master regulator of steroid metabolism, remains unaddressed in the literature, although a strong connection exists between steroidogenesis and sphingolipid metabolism (Lucki and Sewer, 2008). In any case, what is clear is that SF-1 binds several chemically distinct lipid species whose headgroups are structurally exposed to solvent (Fig 2C), and SF-1 needs these lipids for full biological activity.

PIP2 in SF-1 is phosphorylated by IPMK, but not by p110 PI3Ks

The structures of SF-1 bound to phospholipids indicate that the headgroups are solvent accessible (Fig 2). Since PIP2 and PIP3 are important membrane signaling lipids that have failed to yield crystal structures complexed to SF-1, we simulated how these lipids might bind SF-1 (Blind et al., 2012; Sablin et al., 2009). As expected, these simulations revealed the inositol headgroup to be solvent exposed (Fig 3), and led us to hypothesize that PIP2 bound by SF-1 might be accessible to lipid signaling enzymes. The inositol polyphosphate multi-kinase (IPMK) demonstrates this activity, and the kinetics of IPMK activity on SF-1/PIP2 differ greatly from PIP2 in membrane systems (a 6 fold increases in K_{CAT}/K_M, (Blind et al., 2012)), showing IPMK favors phosphorylation of SF-1/PIP2 *in vitro* (Blind et al., 2012). Chemically or genetically impeding IPMK in cells decreases SF-1 gene activation, and is dependent on SF-1 binding to PIPs. The lipid phosphatase PTEN de-phosphorylates PIP3 bound to SF-1, and oppositely regulates SF-1 gene expression in cells compared to IPMK (Blind et al., 2012). Importantly, PI3-kinases of the p110 class (PI3Kα, PI3Kγ and

PI3Kδ) fail to phosphorylate PIP2 bound to SF-1, suggesting that IPMK somehow has unique access to PIP2 held by SF-1, as simulated in figure 4 (Fig 4) using the structures of yeast IPMK (IPK2) and the simulated mouse SF-1 bound to PIP2. These data demonstrate that lipids bound to a non-membrane protein can be modified by lipid signaling enzymes, regulating the biological activity of the protein. Importantly, lipid substrate properties are altered when bound by non-membrane proteins, as p110 PI3-kinases are inactive on SF-1/PIP2 (Blind et al., 2012). The structural elements specific to IPMK that allow phosphorylation of SF-1/PIP2, and/or those of the p110-class of PI3-kinases that prevent activity, await further investigation.

Nuclear Lipid Signaling and SF-1

The physicochemical arrangement of SF-1 as a non-membrane nuclear protein/lipid complex provides a model system that may begin to explain a large body of literature that has documented several interesting biochemical and cell-biological phenomena. The nucleus of eukaryotic cells seems to contain a significant fraction of lipids that do not localize to any known bilayer structure (Follo et al., 2012; Hunt, 2006; Irvine, 2003; Maraldi et al., 1994). These lipids are not only visualized within the nucleoplasm (Barlow et al., 2010; Boronenkov et al., 1998; Hammond et al., 2009) but are also metabolically distinct from membrane lipids (Follo et al., 2013; Keune et al., 2013; Lindsay et al., 2006; Rose and Frenster, 1965; Vann et al., 1997). Further, many lipid signaling and metabolic enzymes shuttle into the nucleus (Bassi et al., 2013; Boronenkov et al., 1998; Schramp et al., 2012), however it is unknown if these enzymes perform the same functions within the nucleoplasm as they perform at cytoplasmic membranes. The simplest explanation of these data is that non-membrane nuclear lipids are bound by soluble nuclear proteins, and that this association alters which enzymes can modify/metabolize them (Barlow et al., 2010; Lindsay et al., 2006). That SF-1 binds phospholipids, and is a soluble, non-membrane protein acted on by lipid signaling enzymes (Blind et al., 2012), begins to shed light on these phenomena. It also provides a model system to further interrogate the signaling functions of nuclear lipids, and to ask what evolutionary advantage signaling enzyme activity on protein/lipid complexes might confer to networks and systems (Poyatos, 2012).

Each SF-1 phospholipid presents unique chemistry to signaling enzymes

Although the kinase activity of IPMK on PIP2 held by SF-1 is new in itself, it raises the interesting question of what advantage phosphorylating a lipid bound to a protein, rather than the protein itself, grants the cell. The new phosphate incorporated into PIP3 may well have evolved to be incorporated into a structurally analogous serine or threonine residue, so why phosphorylate a small molecule associated with a protein rather than the protein itself? The answer may lie with the inherent ability of SF-1 to accommodate several different phospholipid species (Sablin et al., 2009), each presenting a unique chemical interface for signaling enzymes on the SF-1 protein (Blind et al., 2012). An amino acid encoded by the genome would be present in every SF-1 molecule made by the cell (save alternative splice variations). However, by dynamically altering which lipid occupies SF-1, the cell gains the potential to chemically couple and/or decouple SF-1 from particular signaling enzymes, independent of space, time or signaling dynamics.

Phospholipids can chemically decouple SF-1 from particular signaling enzymes

To illustrate this mechanism, take the examples of PIP2 and PC (Fig 2E) bound to SF-1 (Fig 5A). It is well established that both these chemically distinct phospholipids bind SF-1 (and the close SF-1 homolog LRH-1) to regulate gene expression programs (Blind et al., 2012;

Lee et al., 2011; Musille et al., 2013; Musille et al., 2012; Sablin et al., 2009; Sablin et al., 2003). However, kinases like IPMK require a free hydroxyl group to catalyze phosphorylation. Since PC lacks this hydroxyl, it consequently lacks the headgroup chemistry necessary to allow phosphorylation by IPMK (Fig 2E). SF-1 occupancy by PC must therefore decouple SF-1 from direct IPMK signaling, allowing SF-1 activities to occur independent of IPMK kinase activation (Fig 5A). Similarly, PTEN catalyzes de-phosphorylation of phosphorylated inositol lipid species in (Maehama and Dixon, 1999), so when SF-1 is occupied by PC, PTEN signaling is decoupled from SF-1 activity (Fig 5B), enabling the SF-1 circuit to operate independently of PTEN-sensitive branches of the full signaling network. It should also be noted that since PI3-kinases of the p110-class do not act on SF-1/PIP2, SF-1 also represents a signaling molecule that is coupled to PTEN signaling, but inherently de-coupled from p110 PI3-kinase signaling (Blind et al., 2012).

This dynamic responsiveness to signaling based on SF-1 lipid occupancy may not be limited to phospholipid-class lipids. Although only IPMK and PTEN have been demonstrated to act directly on SF-1/lipid complexes, simulations of SF-1 binding other lipids suggest they may act through similar mechanisms (Fig. 6). The sphingolipid signaling enzyme acid ceramidase catalyzes hydrolysis of the unique N-acyl bond in ceramide to generate sphingosine and a free fatty acid (Fig 6A) (Lucki and Sewer, 2012). While only small amounts of ceramide have been found associated with SF-1 in the cell lines that have been examined (Urs et al., 2007), relatively high amounts of sphingosine have been found bound to SF-1, which inhibits SF-1 gene expression programs in cells (Fig 2E) (Urs et al., 2006). Acid ceramidase physically interacts with SF-1 and co-localizes to the nucleus in living cells (Lucki et al., 2012). Since lipids of the phospholipid-class chemically lack the N-acyl bond ceramidases hydrolyze (Fig 2E) (Bhabak et al., 2012), ceramidases cannot act on phospholipids such as PIP2, PIP3 or PC. Phospholipids may therefore decouple SF-1 from direct sphingolipid signaling pathways (Lucki et al., 2012; Urs et al., 2006), enabling the SF-1 circuit to operate independently of that branch of the full network. Diacylglycerol kinase theta (DGKθ) is another lipid signaling enzyme known to generate phosphatidic acid from diacylglycerol (DAG) (Raben and Tu-Sekine, 2008). DGKθ interacts with SF-1 (Li et al., 2007) and DGKθ activity stimulates SF-1 gene expression programs in living cells (Cai and Sewer, 2013). Again, since IPMK requires an inositol headgroup to catalyze phosphorylation (Blind et al., 2012; Resnick et al., 2005), when DAG occupies SF-1, SF-1 must be decoupled from direct IPMK signaling.

Are other nuclear receptors regulated by the same mechanism?

Some other NRs that may be regulated similarly as SF-1 are Liver Receptor Homolog-1 (LRH-1) (Lee et al., 2011; Musille et al., 2013; Ortlund et al., 2005), and the Peroxisome Proliferator Activated Receptor (PPAR) members of the nuclear receptor superfamily (Wahli and Michalik, 2012). LRH-1 is known to bind PIP2 and PIP3 in vitro (Krylova et al., 2005), making IPMK and PTEN activity possible in cells. LRH-1 and IPMK are both highly expressed in the human liver (Chang et al., 2002), and both reside in the nucleus (Resnick et al., 2005), however it remains to be determined if IPMK and/or PTEN directly regulate LRH-1 (Fig 6B).

The PPARs (alpha, beta/delta and gamma) are also nuclear receptors that are well established to bind lipids and be regulated by them (Baker et al., 2005; Issemann and Green, 1990; Wahli and Michalik, 2012). It is also clear PPARs are regulated by many lipid signaling enzymes in living cells (Huang et al., 2011; Pandey et al., 2008; Tsukahara et al., 2010), however the mechanism of that regulation has not been addressed. Two simple mechanisms exist for regulation of PPARs, one of which is commonly assumed to occur - that PPARs dynamically exchange different lipids with membrane systems, not unlike

phosphoinositide transfer proteins. But another non-exclusive mechanism is that lipids bound to PPARs are modified by lipid signaling enzymes while bound to the PPAR protein. For example, phosphatidic acid is known to bind and activate PPARα in cells, and DAG kinase ζ, an enzyme that phosphorylates DAG in membrane systems to generate phosphatidic acid, is also known to activate PPARs (Huang et al., 2011). Thus, DAG kinase ζ may be directly phosphorylating DAG held by PPARs to generate phosphatidic acid within PPAR (Fig 5C). A second example may be Phospholipase D2 (PLD2), a lipid signaling enzyme that generates cyclic phosphatidic acid (cPA) (Tsukahara et al., 2010) from phosphatidic acid in membrane systems, which is known to inhibit PPARγ gene expression (Tsukahara et al., 2010). Could PLD2 be acting directly on PPAR/PA complexes to generate PPAR/cPA? Third, linoleic acid binds PPARα, and is generated by cytosolic Phospholipase A2 (cPLA2) in membrane systems. cPLA2 is known to activate PPAR alpha in cells (Pandey et al., 2008). Should these lipid signaling enzymes prove to act directly on PPAR/lipid complexes, these chemically distinct lipids provide a new mechanism that can connect or de-connect these important transcription factors to lipid signaling pathways, providing specificity to signaling within the network.

How are signaling lipids loaded into non-membrane proteins?

Although lipids regulate the biological activity of the above mentioned non-membrane proteins, there is almost no data describing how these proteins acquire phospholipids from cellular membrane systems. Among many hypotheses, three appear plausible as mechanisms describing how this may happen: direct, entropy-driven phospholipid transfer from membrane systems to the non-membrane proteins, like phosphoinositide transfer proteins (PITPs) (Schaaf et al., 2008); phospholipid-transfer protein mediated exchange between non-membrane proteins (Lev, 2012); and/or co-translational loading of non-membrane proteins at the endoplasmic reticulum.

It is clear that at least some NRs such as SF-1 and LRH-1 are capable of non-enzymatic, entropy-driven phospholipid exchange directly with membrane systems *in vitro* (Blind et al., 2012; Krylova et al., 2005; Sablin et al., 2009), not unlike the phospholipid transfer activities of PITPs (Schaaf et al., 2008). Indeed, the recombinant phospholipid-binding domain of SF-1 could be classified as a phospholipid transfer protein based on its ability to exchange phospholipids between membrane systems *in vitro* (Blind et al., 2012; Cockcroft and Carvou, 2007; Krylova et al., 2005). Phosphoinositide transfer protein (PITP) mediates lipid transfer between membrane compartments (Cockcroft, 2012; Phillips et al., 2006), yet whether or not these same proteins facilitate loading of lipids into non-membrane proteins, or between non-membrane proteins, remains unknown. The Sec14 family of phospholipid transfer proteins is the best characterized family of transfer proteins, with a crystal structure of the human homolog of yeast Sec14 (Sfh1) informing biochemical experiments showing that Sec14 can act as a lipid nanoreactor to facilitate the interaction of particular membrane phospholipids with lipid kinases and phosphatases (Bankaitis et al., 2012; Schaaf et al., 2008). It is important to clarify that whether or not proteins of the nuclear receptor superfamily are capable of dynamic phospholipid exchange with membrane systems in living cells remains an open and very challenging mechanistic question, although it is often simply assumed to occur.

Another way non-membrane lipid binding proteins could acquire lipids is through co-translationally sensing the lipid content at the endoplasmic reticulum (ER, Fig 7). Signal sequences direct mRNA messages that encode secretory and transmembrane proteins to the rough endoplasmic reticulum through the signal recognition particle (SRP). Messages encoding cytoplasmic or nuclear proteins were classically thought to be translated on free ribosomes in the cytosol, independent of SRP and membranes. However, several studies

have used unbiased approaches to demonstrate that the subset of actively translating ribosomal/RNA complexes associated with membranes does not require the presence of a classic signal sequence (Diehn et al., 2000; Lerner et al., 2003; Reid and Nicchitta, 2012). In fact, many members of the nuclear receptor superfamily have been identified as being translated on membrane-bound ribosomes (Diehn et al., 2000), along with many other proteins. One hypothesis explaining this phenomenon is that membrane-translated proteins are co-translationally sensing the ER lipid content by acquiring lipids from the ER as they fold in close proximity to this membrane system. Dissecting how lipids are loaded into non-membrane proteins represents a challenging problem, as lipid exchange is a process that does not chemically alter the substrate, and therefore is difficult to quantify, particularly in living cells (Cockcroft, 1997).

Structural genomics and the future of non-membrane lipid signaling

It was structural analyses that first linked phospholipids to SF-1 (Blind et al., 2012; Krylova et al., 2005; Sablin et al., 2009), and which drove the hypothesis that lipid signaling enzymes might directly act on non-membrane protein/lipid complexes. Other intracellular small signaling molecules have been unexpectedly identified by crystallography in a similar manner, such as Inositol hexakisphosphate (Ins(1,2,3,4,5,6)P6) discovered in the catalytic core of the ADAR2 RNA editing enzyme (Macbeth et al., 2005) and inositol tetrakisphosphate (Ins(1,4,5,6)P4) discovered in the interface between HDAC3 and NCOR2 (Watson et al., 2012). Star-PAP is a nuclear poly-A polymerase that binds PIP2, and whose activity is regulated by PIPK1α to selectively regulate mRNAs (Li et al., 2012; Mellman et al., 2008), however no structural data is available describing the position of the lipid headgroup or its solvent accessibility. Whether or not these complexes are directly coupled to signaling enzymes remains to be determined, but the fortuitous discovery of these small signaling molecules by crystallography highlights the potential of structural genomics to uncover new biology (Telesco and Radhakrishnan, 2012). By applying high-throughput techniques to structure determination we undoubtedly will discover more complexes of small signaling molecules and proteins (Telesco and Radhakrishnan, 2012).

The potential of nuclear protein/lipid complexes to disentangle signaling networks reveals how biology can evolve innovative approaches to take advantage of complex systems like networks, while still maintaining tight regulatory control of individual molecules. The combination of structural genomics to discover new protein/lipid complexes, classic enzymology to demonstrate enzyme activity on these complex substrates, with innovative nuclear & cell biological analyses will provide more insight into signal transduction, nuclear biology and ultimately the design of nature (Poyatos, 2012).

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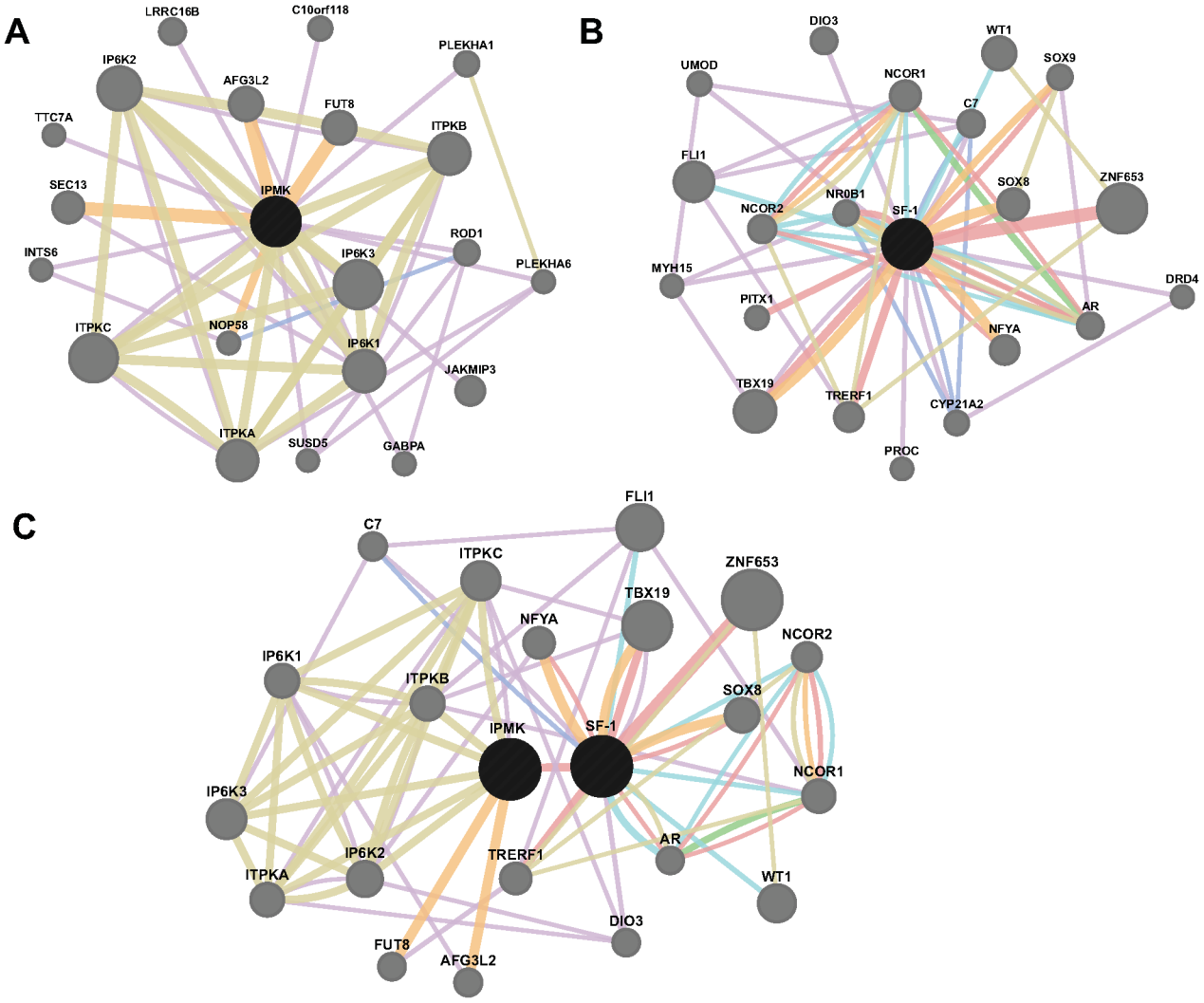

Fig. 1.

Biological networks are highly interconnected. Network interaction maps for the human proteins A) Inositol polyphosphate multikinase (IPMK), B) SF-1, and C. both IPMK and SF-1. Network connections represent Co-expression (Purple), Co-localization (Blue), Genetic interactions (Green), Shared Pathway (Cyan), Physical Interaction (Red), Predicted Interaction (Orange), or Shared Protein Domains (Moss). Maps generated using GeneMANIA (http://genemania.org, University of Toronto).

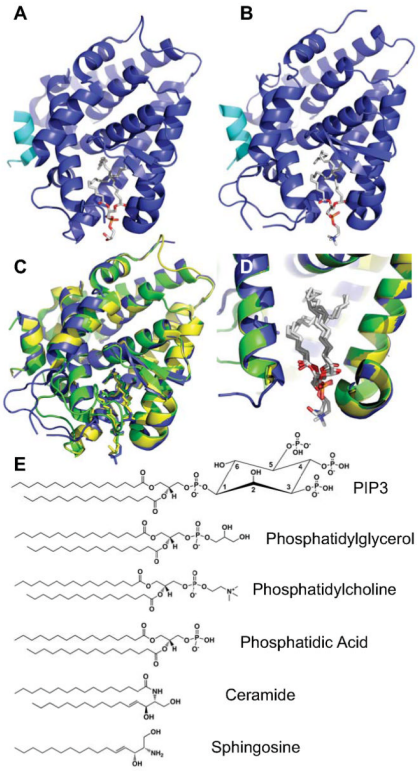

Fig. 2.

SF-1 binds many chemically distinct phospholipid species. A) Crystal structure of mouse SF-1 (Blue), Cofactor peptide (Cyan) and bacterial phosphatidylglycerol (sticks) (PDB: 1YMT). B) Crystal structure of mouse SF-1 bound to eukaryotic phosphatidylcholine (PC), same coloring scheme as A (PDB: 3F7D). C) Superposition of A (Yellow), B (Blue) and mouse SF-1 bound to bacterial phosphatidylethanolamine (Green, PDB: 1YP0). D) Cutaway of C, demonstrating tight superposition of lipids and exposure of lipid headgroups to solvent. E) Several of the lipids known to bind SF-1, demonstrating different headgroup chemistry.

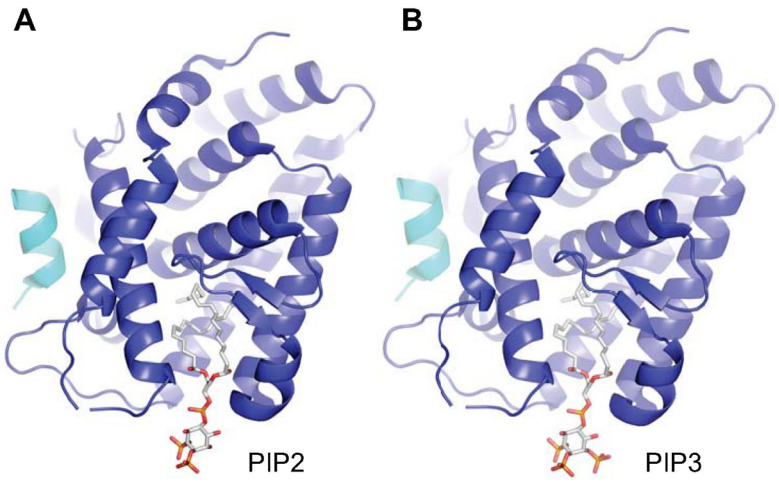

Fig. 3.

SF-1/PIP2 and SF-1/PIP3 simulations suggest a solvent accessible headgroup. A) Simulation of mouse SF-1 bound by PIP2 and B) PIP3, based on mouse SF-1/PC structure (3F7D), predicting high solvent accessibility of lipid headgroups, suggesting they may be available to lipid signaling kinases. Coloring scheme same as Fig. 2.

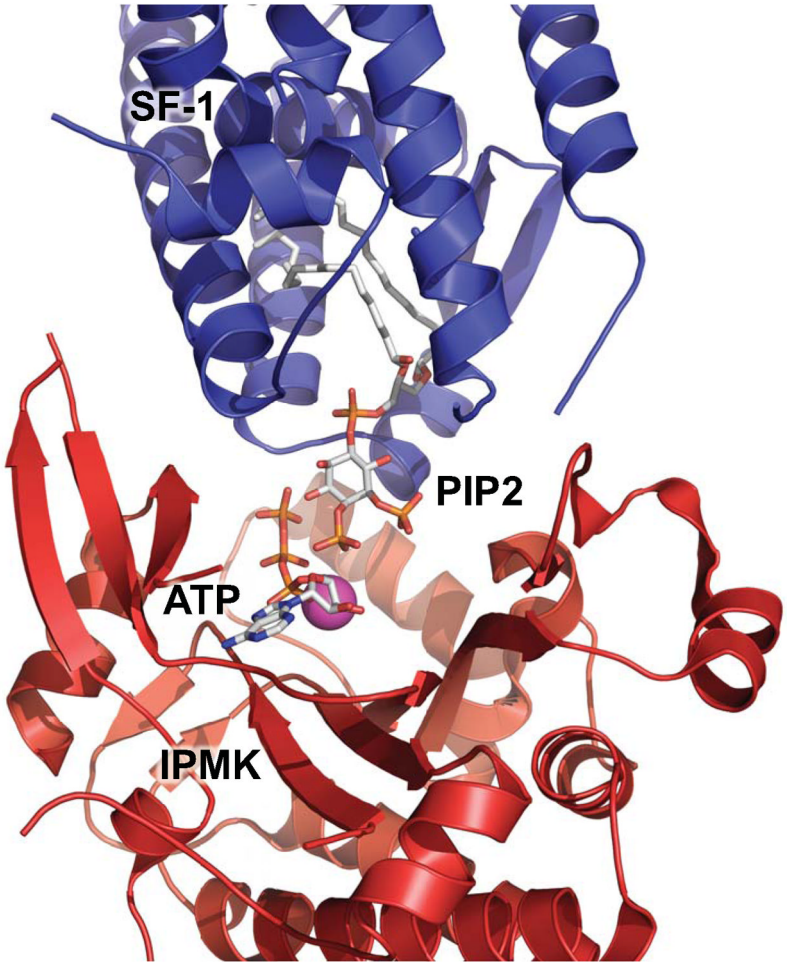

Fig. 4.

Simulation of mouse SF-1/PIP2 interaction with IPMK bound with ATP. Simulation of how mouse SF-1 bound to PIP2 (Blue, simulation from Figure 3A) may interact with yeast IPMK (IPK2, Red PDB: 2IF8), showing bound PIP2 and ATP as sticks, and IPMK-bound magnesium ion (Purple).

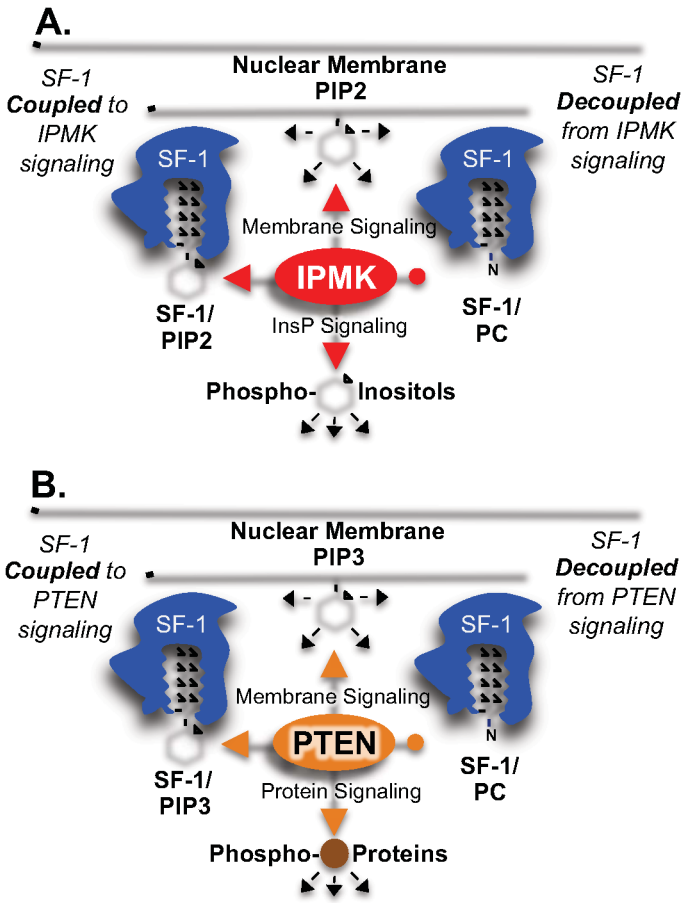

Fig. 5.

Chemically distinct phospholipids can decouple SF-1 from signaling enzymes. A) SF-1 occupied by PIP2 is phosphorylated by IPMK, coupling SF-1 to IPMK signaling. SF-1 occupied by PC cannot be phosphorylated by IPMK, as PC lacks the required headgroup chemistry, de-coupling SF-1 from IPMK signaling. B) SF-1 occupied by PIP3 is de-phosphorylated by PTEN, coupling SF-1 to PTEN signaling, while PTEN has no effect on PC bound to SF-1, decoupling SF-1 from PTEN signaling.

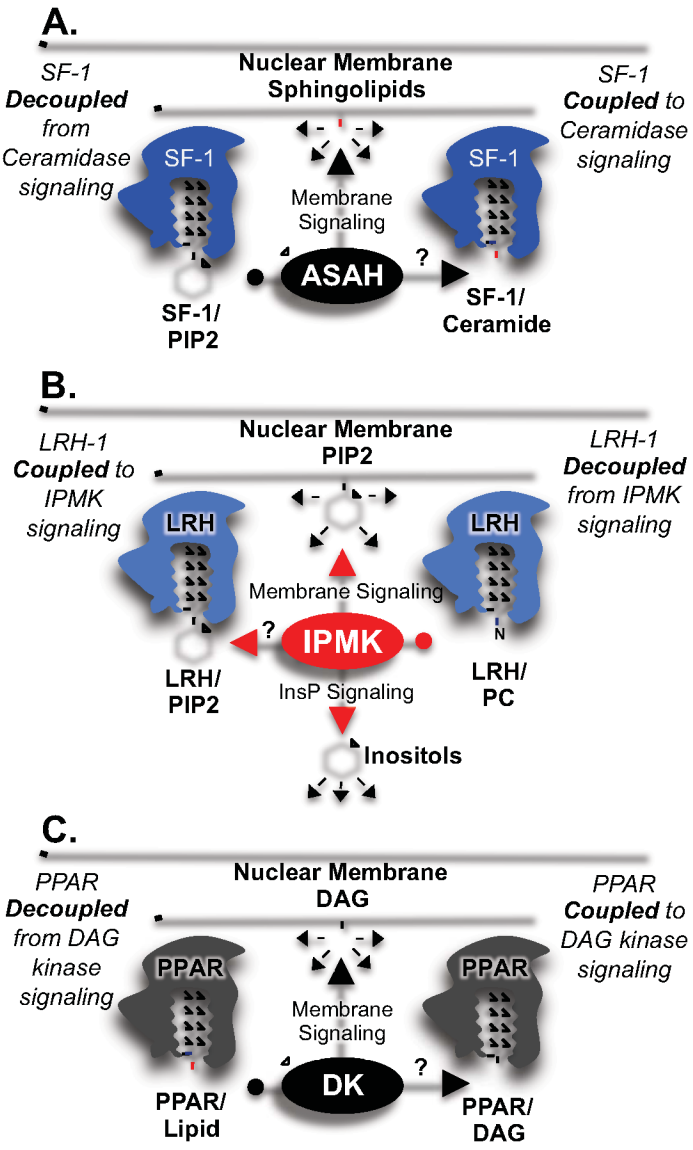

Fig. 6.

Chemically distinct lipids have the potential to decouple other effectors. A) SF-1 occupied by ceramide may be sensitive to ceramidase signaling, but SF-1 occupied by PIP2 will be insensitive, decoupling SF-1 from ceramide signaling. B) LRH-1 occupied by PIP2 may be sensitive to IPMK/PTEN signaling, but LRH-1 occupied by PC will be insensitive. C) PPAR occupied by DAG may be sensitive to DAG kinase signaling, PPAR occupied by other lipids would not.

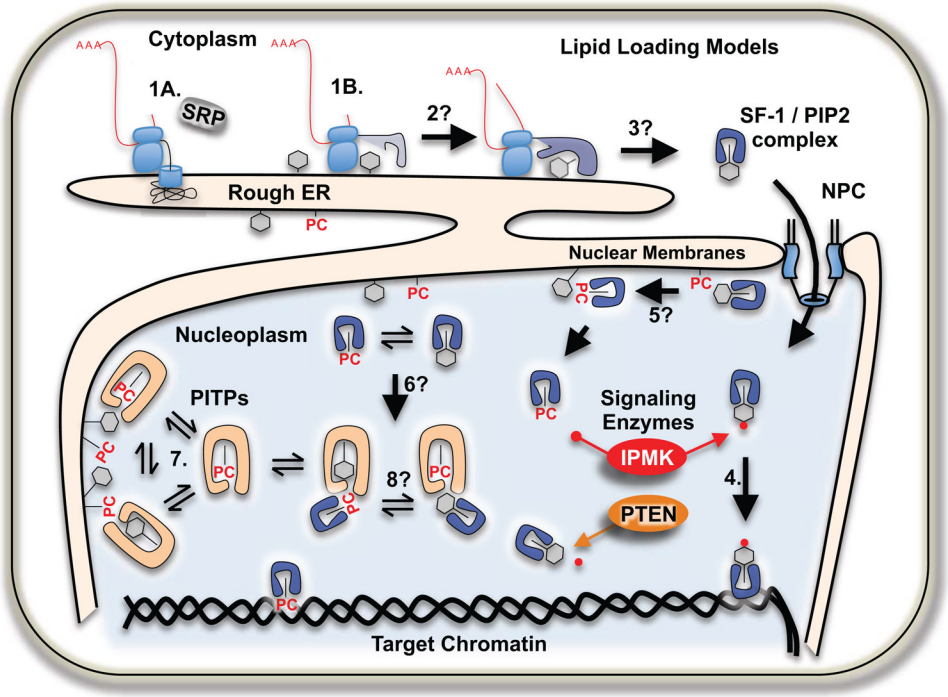

Fig. 7.

Models of lipid loading into non-membrane proteins, like SF-1. Steps labeled with "?" are not known to occur, all other steps are well established to occur. Classically, 1A) only signal-containing nascent peptides are translated on rough ER, however 1B) many nuclear proteins are translated on membrane-bound ribosomes, where 2) folding nuclear proteins may sense the ER lipid content while attaining mature structure 3) then carry that information into the nucleus 4). SF-1 is able to exchange lipids with membrane systems *in vitro* 5) this may happen in cells, although no data exist. Equilibrium concentration fluctuations in membrane lipids 6) may allow varying sensitivity to signaling enzymes such as IPMK and PTEN. Phosphoinositide transfer proteins 7) mediate lipid movement between membrane systems, but it is not known if they aid in loading lipids into non-membrane proteins, aiding in equilibration 8).