Published in final edited form as:

Bioessays. 2018 June ; 40(6): e1700219. doi:10.1002/bies.201700219.

A link between Alzheimer's and type II diabetes mellitus? Ca^{2+} -mediated signal control and protein localization

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Abstract

We propose protein localization-dependent signal activation (PLDSA) as a model to describe pre-existing protein partitioning between the cytosol, and membrane surface, as a means to modulate signal activation, specificity, and robustness. We apply PLDSA to explain possible molecular links between type II diabetes mellitus (T2DM) and Alzheimer's disease (AD) by describing Ca^{2+} -mediated interactions between the Src non-receptor tyrosine kinase and p52Shc adaptor protein. We suggest that these interactions may serve as a contributing factor to disease development and progression. In particular, we propose that signaling response is regulated, in part, by Ca^{2+} -mediated partitioning of lipid-bound and soluble forms of Src and p52shc. Thus, protein-protein interactions that drive signaling in response to extracellular ligand binding are also mediated by partitioning of signaling proteins between membrane-bound and soluble populations. We propose that PLDSA effects may explain, in part, the evolutionary basis of promiscuous protein interaction domains and their importance in cellular function.

Keywords

biological signaling; Src; ShcA; Alzheimer's disease; type II diabetes mellitus; protein-lipid interaction; protein localization

1. Introduction

AD is increasingly referred to as “type III diabetes” because of similarities in the clinical and histological hallmarks between AD and T2DM.^[1, 2] These hallmarks include altered cell mass, deposition of protein aggregates, insulin resistance, chronic inflammation, and hyperglycemia.^[3–5] Shared clinical features suggest common molecular underpinnings of

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Conflict of Interest

The authors declare no conflict of interest.

disease development and progression. At the cellular level, previous studies have demonstrated changes in a wide array of processes including protein synthesis/turnover, energy metabolism, response to oxidative stress, and protein quality control.^[2, 5] Thus, developing effective therapies that target each of these disparate processes is a challenge. In addition, some of these cellular changes are compensatory in nature and not upstream determinants of disease onset or progression. However, it has become increasingly clear that aberrant signaling cascade activity (*e.g.*, the insulin growth factor (IGF), tumor necrosis factor- α (TNF- α), PI3K/AKT, and MAPK pathways) is a key determinant of AD and T2DM onset and progression.^[1, 2, 4–6] Molecular linkages between these disease states via signaling cascade activity are not well defined.^[1, 2, 4–6] Identifying molecular mechanisms that link AD and T2DM is challenging yet may prove a viable avenue for developing efficacious therapeutics that target both disease states.

Towards this end, one may envision signaling cascade activity not as a defined set of static interactions between stable protein populations but as a malleable network of dynamic populations in constant flux. Thus, disruption of protein partitioning between intracellular populations (*e.g.*, membrane-bound and soluble forms) may modulate cascade activity and crosstalk in beneficial ways to treat various human diseases. Here, we discuss the importance of such protein partitioning in the activation of biological signaling that may offer a possible explanation for a link between AD and T2DM. To demonstrate this point, consider generation of membrane action potentials in Schaffer collateral CA1 synapses in the hippocampus and pancreatic β cells. As pointed out by Ederhard, these two cells share common features although they develop from different germ layers.^[7] We propose that by looking into common molecular events in these two different cells such as Ca^{+2} -mediated biomolecular interactions, the molecular basis for AD susceptibility in T2DM patients may be found. We discuss one such example for molecular events involving non-receptor tyrosine kinase (nRTK) Src and its binding partner, p52shc adaptor protein.^[8, 9] Although both Src and p52Shc do not contain conventional Ca^{+2} -binding domains, we propose that their function is regulated indirectly by Ca^{+2} -mediated lipid re-organization in membranes. In this view, fluctuation in intracellular Ca^{+2} concentration determines the amplitude and duration of biological signal propagation by modulating partitioning of SH2 domain-containing proteins between the cytosol and the membrane surface. It follows that the intracellular Ca^{+2} concentration regulates the activity and localization of SH2 domain-containing proteins such as Src and p52shc. Thus, in this model, Ca^{+2} -mediated signaling activation at the membrane surface is a key linkage between AD and T2DM.

Finally, we discuss the evolution of mammalian biological signal transduction in which promiscuous protein-protein interaction (PPI) modules, such as SH2 domains, were created to facilitate partitioning or compartmentalization of biological signals to compensate for increased cellular volume during evolution. We envision the outcomes of biological signal activation to be protein partitioning-dependent. This protein localization-dependent signaling activation (PLDSA) hints at a new drug design strategy to ameliorate chronic diseases.

2. Current model of biological signaling as bow tie-shaped protein-protein interaction (PPI) network

Because aberrant regulation and activation of signaling cascades are known to drive formation of human disease, understanding the dynamic behavior of signal transduction should help identify disease-associated PPIs. Biological signaling networks have been described as having a bow tie-shaped topology in which signaling molecules are classified in three layers with interconnected positive and negative feedback loops (Fig. 1).^[10, 11] In this model, the input layer consists of extracellular ligand-receptor interactions that propagate biological signals to the central core process including kinases, Ras, phosphoinositides (PIs), and Ca^{+2} . The central core proteins communicate with transcription factors in the output layer of the bow-tie network to effect cellular responses. Ebisuya *et al.* proposed ERK-mediated signaling to have such a bow-tie network that leads to distinct cellular responses depending on ERK compartmentalization^[12]: ERK activation due to receptor-ligand interactions (input) causes its dissociation from MEK, causing ERK (core) to translocate to the nucleus. ERK phosphorylates various transcription factors (output) in the nucleus, leading to proliferation and differentiation of PC12 cells. On the other hand, Sef binding to MEK keeps ERK in the cytosol, leading to phosphorylation of Ca^{+2} -calmodulin-dependent MLCK (myosin light chain kinase) by ERK and cell migration in FG-carcinoma cells.^[12]

The bow-tie model tells us that the input layer, receptor-ligand interactions, determines the functional outcome of the central core proteins.^[11–13] However, the central core must play a more significant role in determining cellular fate, considering that kinases can be activated independently of ligand-receptor interactions to modulate cellular response. Indeed, reagents that alter membrane lipid organization are known to activate kinases in a receptor-independent manner including: Syk kinase by monosodium urate, p38MAPK by sphingosine, and c-Src by saturated fatty acids.^[14, 15] The activation of signaling cascades in these receptor-independent pathways must be mediated by changes in the kinase partitioning between the cytosol and plasma membrane surface. We propose that such protein partitioning offers additional mechanisms for determining the type and duration of activated biological signaling cascades. This point is signified by recent findings in which classical PPI domains (e.g., SH2 domains) were shown to bind to negatively charged lipids as described in the following section.^[16]

3. SH2 domain as a Membrane Binding Module

The human genome encodes 111 SH2 domain-containing proteins including kinases, phosphatases, and adaptor proteins.^[17] The role of SH2 domains as a phosphotyrosine binding module is well known and reviewed extensively elsewhere.^[17, 18] However, the molecular basis of SH2 ligand recognition remains ambiguous as SH2 domains display promiscuous binding to a diverse array of phosphorylated proteins and peptides. How do promiscuous PPIs activate specific signaling cascades? In other word, how do SH2 domains select ligands, via either specific or non-specific binding interactions, to elicit appropriate biological responses? One possible solution is limiting the available PPI search space (e.g., compartmentalization or localization) for SH2 domain containing proteins and their cognate

ligands. Previous work has demonstrated the presence of positively charged surface regions on SH2 domains that are distinct from known phosphopeptide/protein binding sites.^[16] These cationic surface patches may provide an interaction surface for negatively charged lipids in membrane bilayers and, as a result, facilitate sequestration of the SH2 domain-containing protein to plasma or organelle membrane surfaces. A recent study by Park *et al.* examined such interactions between negatively charged phosphoinositides (PIs) and 76 human SH2 domains by surface plasmon resonance (SPR).^[16] The study demonstrated that most SH2 domains bind to the plasma membrane (PM)-mimetic vesicles containing PIs with submicromolar affinity. The PI binding affinity was found to be correlated with “shallowness” of the cationic patch: SH2 domains with a deeper cationic groove had higher affinity to PIs relative to SH2 domains with flat or shallow cationic surface patches.^[16] Thus, one could argue that electrostatic interaction between SH2 domains and anionic PIs function to reversibly regulate protein partitioning between the cytosol and membrane surface, depending on membrane lipid composition. This, in turn, may play an important role in SH2 domain ligand selection by restricting the available pool of protein binding partners. Alternatively, anionic lipid binding may allosterically modulate SH2 domain interactions with phosphoprotein substrates, contributing to selective activation of biological signaling.

SH2 domain-containing proteins may only transiently remain at the membrane surface due to membrane fluidity. Metal cations, particularly Ca^{+2} , are known to facilitate the formation of anionic lipid clusters in membranes by bridging lipid head groups.^[19] Thus, intracellular $[\text{Ca}^{+2}]$ may be important for spatiotemporal regulation of protein partitioning and subsequent signaling activation. In the following sections, we describe the potential interplay between intracellular $[\text{Ca}^{+2}]$ and anionic lipids in regulating the function of the canonical nRTK, Src, and its binding partner, adaptor protein p52shc, as an example for protein localization-dependent signal activation (PLDSA).

4. How does an interplay between Ca^{+2} oscillation and protein localization impact biological signal transduction?

The nRTK Src is activated by autophosphorylation, leading to tyrosine phosphorylation of Src substrate proteins.^[9] In addition to the C-terminal kinase domain that contains the Tyr autophosphorylation site to regulate its activity, Src consists of SH3 and SH2 domains that bind proline-rich and phosphorylated protein ligands, respectively.^[9] Although both SH3 and SH2 domains are considered as protein-interaction domains in the conventional model of biological signal transduction,^[20] recent studies reported their additional functional roles as anionic lipid binding domains^[16, 21], as described in the previous section. The Src SH3 domain also interacts with negatively charged phospholipids such as phosphatidylserine (PS) and phosphatidic acid (PA).^[21] Tight packing of these lipids in membranes is facilitated by metal cations, such as Mg^{+2} and Ca^{+2} , by bridging the charged lipid head groups, forming microdomains in the plasma membrane.^[19] In particular, Ca^{+2} plays a major role in microdomain assembly and disassembly since intracellular $[\text{Ca}^{+2}]$ fluctuates roughly between 100 nM and 1 mM.^[22] This presents the question, how does interaction between Src and anionic lipid microdomains impact activation of downstream biological signaling

cascades? Ca^{+2} -mediated lipid clustering should facilitate Src localization via SH3 and SH2 domain binding and formation of signaling complexes at the membrane surface, sustaining an activated biological signal. In addition to regulating the duration of biological signal propagation, the type of activated signaling pathway can be determined by lipid-Src interactions. For example, SH3 domain occlusion due to membrane binding promotes binding interaction between the Src SH2 domain ligand binding. One example of this is SH2 domain binding to p130^{Cas}, an adaptor protein involved in the MAPK signaling pathway.^[23] On the other hand, SH2-mediated lipid binding will promote interaction between the SH3 domain and proteins containing proline-rich regions such as cytoplasmic domains of the progesterone and integrin receptors.^[24] Thus, one can argue that while Ca^{+2} directly acts on various Ca^{+2} binding proteins such as protein kinase C (PKC) and phospholipase C (PLC) to activate signaling pathways^[25], proteins devoid of canonical Ca^{+2} binding modules such as EF-hand and C2 domains should also be activated at the membrane surface by Ca^{+2} -mediated anionic lipid localization. Therefore, the magnitude of Ca^{+2} oscillation, occurring on a millisecond to second timescale,^[26] may have a profound effect on transient or sustained activation of biological signaling by modulating the partitioning of SH2 domain-containing proteins between the cytosol and membrane surface.

5. Solution environment influences signaling cascade activity by determining the location and abundance of p52Shc tyrosine phosphorylation

Adaptor proteins also play a major role in regulating biological signaling. Indeed, kinase-dependent phosphorylation of signaling adaptor proteins is a key determinant in assembling signaling protein complexes. One such example is the p52Shc adaptor protein, which binds c-Src, and regulates the EGFR, Ras/MAPK, and GPCR-mediated signaling pathways.^[27, 28] Like Src, p52shc is a multi-modular domain protein containing protein interaction domains such as the N-terminal phosphotyrosine binding (PTB), the central collagen homology-2 (CH2), and C-terminal SH2 domains.^[28] Like SH2, the PTB domain also binds to anionic PIs,^[29] suggesting the importance of p52shc function in sequestering binding partners to the membrane surface enriched with anionic lipids. In this regard, p52shc shifts from playing a passive scaffolding role to an active participant of signaling pathways by determining the cellular localization of protein binding partners in a Ca^{+2} -dependent manner.

Studies conducted *in vitro* demonstrated that Src and p52Shc lipid binding activity modulate the abundance, and site, of tyrosine phosphorylation in the p52Shc substrate protein.^[30] Using a membrane-mimetic solution environment containing a nonionic detergent, octyl glucose neopentyl glycol (OGNG), Src robustly phosphorylates specific tyrosine residues (Tyr 239/240) in p52shc that recruit an adaptor protein (Grb2) involved in MAPK signaling pathway activation.^[30, 31] This result implies that Src and p52Shc membrane localization enhances Src activity and MAPK-associated tyrosine phosphorylation in p52shc. In contrast, in the absence of OGNG or in the presence of phosphatidylinositol-4-phosphate without Ca^{+2} , p52Shc was phosphorylated at residues associated with interleukin-2 (IL-2) signaling cascade activation.^[30, 32] These findings suggest that protein partitioning determines the

type of activated signaling cascades by directing specific phosphorylation of adaptor proteins that subsequently leads to selective recruitment of downstream signaling proteins.

6. Intracellular Ca^{+2} concentration coordinates the duration and termination of biological signal transduction

Because Ca^{+2} oscillation occurs on a timescale of milliseconds to seconds,^[26] one can argue that Ca^{+2} impacts the timescale of anionic lipid microdomain assembly and signaling complex formation/activation at the membrane surface. Indeed, calcium oscillation may serve as a vital link between microdomain assembly and the duration, and type, of signal transduction. Here, we describe a molecular scenario, using the Src kinase and p52shc adaptor protein interaction as an example, for Ca^{+2} -mediated protein localization-dependent signal activation (PLDSA) within a cellular context. In particular, we focus on interesting similarities in Ca^{+2} sensitive molecular response between pancreatic β and postsynaptic cells to highlight differences in the type of signaling response depending upon Src and p52Shc subcellular localization.

In both pancreatic β and postsynaptic cells, Ca^{+2} influx occurs following receptor-ligand interactions.^[33, 34] When receptors are not activated, Src partitions between the cytosol and the membrane surface (Fig. 2a). Because neuronal Src, n-Src, contains a unique segment similar to a cholesterol binding CARC motif,^[35] the predominant population of n-Src is located at the membrane surface (Fig. 2a). In neurons, NMDAR binding to glutamine or glycine produces Ca^{+2} influx into postsynaptic cells leading to increased intracellular $[\text{Ca}^{+2}]$ (Fig. 2b).^[33] NMDAR activation correlates with Src-mediated phosphorylation of NMDAR cytosolic domains, a process that plays a crucial role in inducing and maintaining long-term potentiation (LTP) for learning and memory consolidation.^[36, 37] In pancreatic β cells, Ca^{+2} influx occurs as a result of glucose transport via glucose transporters and subsequent opening of voltage-dependent calcium channels in the plasma membrane (Fig. 2b). This leads to insulin secretion from the β cell.^[34] In both instances, activation of the PI3K/AKT and MAPK signaling pathways may be mediated by Ca^{+2} -induced anionic lipid clusters at the membrane surface in both CA1 pyramidal neurons and pancreatic β cells (Fig. 2b). In neurons, PI3K/AKT and MAPK activation is important in synaptic plasticity and LTP, respectively.^[38, 39] In pancreatic β cells, PI3K/AKT activation stimulates glucose transport and glycogen synthesis while MAPK activation leads to transcription of genes involved in cell proliferation.^[40]

We propose that amplification and duration of the above signaling responses is driven, in part, by protein partitioning in response to the magnitude and length of Ca^{+2} oscillation. Transient increases in intracellular $[\text{Ca}^{+2}]$ may stimulate response pathways predominantly in the cytosol instead of at the membrane surface. Proteins that are activated by cytosolic increases in $[\text{Ca}^{+2}]$ include other kinases such as protein kinase C (PKC) and calcium-regulated non-receptor proline-rich tyrosine kinase β , both of which are known cytosolic Src activators.^[37, 41]

Because p52shc also has lipid binding PTB and SH2 domains, its localization and phosphorylation are also dependent on intracellular $[\text{Ca}^{+2}]$. Phosphorylation of p52shc by c-

Src in the cytosol activates IL-2 signaling, deficiency of which is associated with attenuation of T-cell function, leading to immune intolerance and pancreatic β cell dysfunction.^[30, 32] In neurons, IL-2 was shown to restore T-cell function and alleviate AD symptoms including the loss of synaptic plasticity and memory.^[42] Thus, when Src and/or p52shc are away from the membrane, it appears to offer protective effects against disease-associated molecular events.

In the above model, termination of biological signaling at the membrane surface is associated with reduced intracellular $[Ca^{+2}]$ and concomitant dispersion of anionic lipid clusters that increases the cytosolic pool of both p52shc and Src (Fig. 2c). Thus, we speculate that Ca^{+2} buffering by Ca^{+2} binding proteins such as parvalbumin, calmodulin, and calreticulin as well as Ca^{+2} reuptake by the sarco/endoplasmic reticulum Ca^{+2} -ATPase impacts the activation of proteins containing SH3, SH2, and PTB domains. It then follows that chronic elevation of Ca^{+2} preferentially activates signaling cascades at the membrane surface that may be associated with pathological conditions.

7. A proposed model for the development of AD and T2DM

In pathological conditions including AD and T2DM, intracellular $[Ca^{+2}]$ is chronically elevated.^[43, 44] Under such a condition, Src and p52shc are expected to accumulate at the membrane surface by binding to anionic lipid clusters via SH3, SH2, or PTB domains (Fig. 2d). Src recruitment to the membrane surface is associated with the activation of stress-induced kinase, c-Jun N-terminal kinase (JNK), in NIH3T3 cells stimulated with saturated fatty acids.^[15] This is a pathological molecular event as increased saturated fatty acids in membranes are found in AD and T2DM patients.^[15, 45] In this disease model, elevated saturated fatty acids together with Ca^{+2} causes Src and JNK to co-localize at the membrane surface, leading to hyper-phosphorylation of insulin receptor substrate-1 (IRS-1) and attenuation of AKT activity (Fig. 2e). Reduced AKT activity is associated with the development of metabolic syndrome such as hyperglycemia and hypertriglyceridemia, both risk factors for T2DM.^[44, 46] In neurons, JNK activation is associated with tau hyperphosphorylation and neuronal loss, hallmarks of AD in humans.^[39, 47] Because of chronic elevation of intracellular Ca^{+2} , signaling complexes are stabilized at the membrane surface, resulting in sustained signaling cascade activation (Fig. 2f).

These molecular scenarios suggest that combination drug therapy that restores Ca^{+2} homeostasis and inhibits signaling molecule function may be an effective treatment strategy. For example, the administration of memantine, an NMDAR antagonist, and cholinesterase inhibitor showed a promising effect in preventing the progression of AD.^[48] This observation suggests that combination therapies including the management of aberrant Ca^{+2} homeostasis could offer beneficial effects by reducing membrane-associated signaling activation. Such an approach may be expanded to include kinase inhibitors to treat diverse AD and T2DM symptoms.

8. Pre-existing protein partitioning determines biological signal responsiveness

The bow tie signaling architecture is postulated to explain underlying design principles of biological signaling.^[13, 49] nRTKs, including c-Src, are a subset of the central core in the bow-tie EGFR signaling structure.^[50] In particular, reversible nRTK regulation by phosphorylation is the key to generate robust, yet flexible, biological responses to external stimuli.^[50] We propose that protein partitioning, such as in the c-Src-p52shc interaction, underlies another means by which signal response and duration is determined.

To illustrate this point, we consider the mixture of various conformers, or a protein ensemble (Fig. 3). Because proteins exist in an ensemble of various conformers,^[51] the activation of signaling cascades can be envisioned as a redistribution of conformers within the ensemble. For example, because intracellular ATP is always present, one would anticipate a pre-existing population of active (auto-phosphorylated) and inactive (unphosphorylated) kinase in a basal state (Fig. 3a,d). Upon receptor activation, the inactive kinase population undergoes autophosphorylation, and the active kinase population interacts with its binding partner (Fig. 3b,e), causing depletion of free active kinase (Fig. 3c,f, dotted red sphere). Figure 3 depicts a scenario where fewer conformers in a pre-existing active state (Fig. 3d) leads to a sudden increase in the newly bound population (Fig. 3f, red circle), or the production of robust biological signal, relative to a slowly activated system (Fig. a–d). Thus, we speculate that responsiveness of a biological system depends on pre-existing conformers or pre-partitioning of central core processing proteins. Termination of a biological signal requires replenishment of the inactive form (Fig. a,d). This can be accomplished via re-establishing the pre-existing equilibrium by dephosphorylating the active form or by synthesizing protein. This simple model illustrates that a robust system (Fig. 3d–f) requires more efficient restoration mechanisms, relative to a slowly activated system (Fig. a–c), to populate the pre-existing inactive form and return to a basal state (Fig. 3a,d). It follows that a robust system may be more sensitive to defects in restoration mechanisms, including phosphatase and protein synthesis machinery. Conversely, the slowly activated system (Fig. a–c) can be re-activated more readily relative to the robust system (Fig. d–f). This suggests that a slowly activated system may be more susceptible to chronic activation. Applying these principals to the p52Shc/c-Src system discussed above, the pathological state is associated with a strong equilibrium shift to membrane-bound p52Shc and c-Src. This state is best described by the slowly activated system (Fig. 4a–c), which can be more readily re-activated relative to the robust system (Fig. 4d–f). This simple illustrates that changing the pathological pre-partitioning state (Fig. 4a) towards the cytosolic form, as in Fig. 4d, offers a possible therapeutic strategy.

The p52Shc/c-Src system serves as a specific example of modulating protein localization (*i.e.*, pre-existing protein pools) via Ca^{+2} -mediated lipid microdomain formation within the membrane. We would argue that other molecules, such as ATP, are also expected to impact pre-existing partitioning of central core proteins (*e.g.*, kinases) to shift the overall distribution towards more active, or inactive, proteins. As such, one would envision that the outcomes of biological signaling events are tightly coupled to cellular metabolism.

9. A possible contribution of macromolecular crowding to the appearance of promiscuous domains during evolution

The bow-tie model envisions signaling as a binary switch (on/off) within the framework of a tightly regulated PPI network. One can also envision signaling, in some systems, using the protein localization-dependent signal activation (PLDSA) model in which protein partitioning determines the type, and duration, of activated biological signaling pathway activity. The idea that localization controls protein activity is not a novel concept in itself though an integral component to the current PLDSA model is the role that promiscuous binding domains, such as SH3 and SH2 domains, play in driving pathway activation (as demonstrated above with the p52Shc and c-Src system). In contrast to mammalian signaling, bacterial signaling consists of two-component signaling (TCS) involving specific phosphoryl transfer from histidine kinases (HKs) to receiver proteins.^[52] We speculate that this system difference arises from macromolecular crowding: the intracellular concentrations of biomolecules are higher in *E.coli* compared to mammalian cells. This corresponds to an ~8 and ~3 times slower protein translational diffusion, respectively, relative to water.^[53] Increased crowding within the bacterial cytoplasm creates aqueous phase separation,^[54] that should effectively compartmentalize TCS, allowing rapid signal transduction. We speculate that the expansion of cellular volume (decreased macromolecular crowding) during evolution demanded components that localize PPIs in biological signaling. This evolutionary pressure is one contributing factor for the appearance of promiscuous SH3 and SH2 domains with lipid binding activity: weak PPIs should allow protein localization at the membrane surface while avoiding unwanted and tight protein interactions in the cytosol. Weak PPIs also facilitate transient and strong signaling activation that underlies robust biological responses.^[55] Protein partitioning results in the production of multiple responses by activating signaling in the cytosol and at the membrane surface. In other words, unlike TCS, an input signal is “diluted” by protein partitioning to generate multiple biological responses including gene transcription, protein synthesis, and metabolism that allow organisms to make time- and energy-efficient adjustments, depending on external perturbations. In this regard, a linearly interacting PPI, as in bacterial TCS, may even represent pathological conditions in mammalian cells.

Approaching biological signaling within the framework of a PLDSA model may further augment how one pursues development, and application, of treatment strategies for human disease. While conventional orthosteric drugs aim to turn off the activity of a target protein, PLDSA suggests altering the pre-existing equilibrium partitioning of a therapeutic target may also impact signaling cascade activity. Such a therapeutic approach would include modulating conformation and cellular localization of central core proteins including kinases. If PPI at the membrane surface is associated with a disease (Fig. 2), can compounds be developed that modulate protein conformations to increase cytosolic pools and, as a result, decrease the membrane-bound population? Alternatively, can compounds be developed that modulate membrane lipid composition to dissociate specific protein populations from the membrane surface? The development of such compounds, together with the management of Ca^{+2} homeostasis, could be an intriguing addition to future drug design strategies.

Conclusions and Future Directions

Protein domains that engage in promiscuous interactions play an important role in molecular crosstalk and protein partitioning between cytosolic and membrane-bound pools. These domains evolved under a selective pressure that disfavored static protein localization and long-term activation of downstream signaling cascades. Two proteins with promiscuous binding domains, the Src kinase and p52Shc adaptor protein, serve as examples to demonstrate this point. In our model, and as supported by available data, intracellular $[Ca^{+2}]$ functions to modulate localization of these proteins between cytosolic and anionic membrane microdomain interacting forms. We have presented an argument in which altering these two pools (shifting to/from membrane-bound form) will impact downstream signaling cascade activity and molecular crosstalk. Thus, it is this dynamic redistribution that may explain, in part, molecular underpinnings of pathological conditions such as AD and T2DM. Furthermore, we propose that PLDSA may explain, in part, the evolutionary basis for promiscuous protein interaction domains and their role in cellular function. Further studies are required to define more clearly the impact of protein localization on activity, and crosstalk, of downstream signaling cascades.

Acknowledgments

This work was supported by funding from the National Institute of General Medical Sciences from the National Institutes of Health under grant number R01GM118599 (Hays).

Abbreviations

AD	Alzheimer's disease
T2DM	type II diabetes mellitus
PPI	protein-protein interaction
CARC	cholesterol amino acid recognition consensus
SERCA	sarco/endoplasmic reticulum Ca^{+2} -ATPase
CAKβ	calcium-regulated non-receptor proline-rich tyrosine kinase
PS	phosphatidylserine
PA	phosphatidic acid
IRS-1	insulin receptor substrate-1
JNK	c-Jun N-terminal kinase
PI3K/AKT	Phosphatidylinositol-4,5-bisphosphate 3-kinase/Protein kinase B
TCS	two-component signaling

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Figure 1. Bow-tie signaling model

Protein-protein interaction (PPI) networks in biological signaling are proposed to have an hourglass or bow-tie topology. Biological signals produced by receptor-ligand interactions (shown in orange circles) are propagated and integrated by the central core processes including kinases and Ras (shown in cyan circles). The signals are then propagated to various transcription factors (shown in green circles) to produce biological responses. Black lines connecting the circles represent interactions. Positive (red arrow) and negative (blue arrow) feedback loops define behavior of the signaling systems. Negative feedback loop consists of phosphatases, GTPases, and receptor endocytosis.

Figure 2. Model for Ca^{2+} -mediated biological signal control

Proposed Ca^{2+} -mediated molecular events in healthy (a–c) and pathological (d–f) states. (a) In the receptor (channel) inactivated state, Src partitions between the cytosol and the membrane surface. In postsynaptic cells, predominant population of n-Src may be associated with the membrane surface via cholesterol (shown in grey spheres) binding. (b) Upon receptor activation, Ca^{2+} (shown in brown spheres) influx occurs followed by Src activation via autophosphorylation (phosphorylation shown in red sphere with "P"). This facilitates the formation of anionic lipid microdomains and recruitment of Src to the membrane surface. Activated Src phosphorylates NMDAR in postsynaptic cells. Src is also involved in the assembly of signaling complexes as shown in the figure. (c) Reduced intracellular Ca^{2+} causes the dispersion of the anionic lipid microdomains and dissociation of signaling complexes, terminating biological signaling. (d) Chronic elevation of intracellular $[\text{Ca}^{2+}]$ in AD and T2DM causes Src to localize at the membrane surface by forming the anionic lipid microdomains (shown in purple spheres) in the receptor (channel) inactivated state. (e) Further increase in $[\text{Ca}^{2+}]$ upon receptor activation facilitates formation of additional microdomains (shown in red spheres). Src is involved in formation of signaling complexes as shown in the figure. (f) Due to defects in maintaining Ca^{2+} homeostasis, intracellular $[\text{Ca}^{2+}]$

⁺²] cannot be effectively buffered, causing the signaling complexes to remain at the membrane surface, leading to chronic activation of biological signaling.

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Figure 3. Relationship between pre-existing protein partitioning and biological signal robustness Blue and red spheres are inactive, unphosphorylated and active, phosphorylated populations, respectively. A binding partner in a downstream signaling cascade is shown in brown squares. In this model, a fully activated system consists of five bound complex. A signaling system is activated in a steady (a–d) or robust (e–h) manner. (a,e) There are pre-existing active and inactive population in a basal state. In this model, the bound form is assumed to be more stable than the free active form. (b,f) Upon receptor activation, the inactive form undergoes autophosphorylation, populating the free active form. (c,g) The free active form binds to its binding partner immediately after auto-phosphorylation, generating a newly formed bound population (shown in red circle). This causes depletion of the free active form (shown in dotted-red spheres).

Figure 4. Model for disease-associated partitioning for Src and p52shc interaction

Membrane-bound and cytosolic Src/p52shc is shown in red and cyan spheres, respectively. In this model, a fully-activated state consists of 6 red spheres. For clarity, protein is assumed to localize at the membrane surface upon the activation of biological signal although the active state can also stay in the cytosol as discussed in the text. **(a–c)** A pathological state with chronic Ca^{+2} elevation **(d–f)** A healthy state with normal Ca^{+2} homeostasis **(a)** Because of chronic elevation of Ca^{+2} , the more membrane-bound form populates than **(d)** the state with normal Ca^{+2} homeostasis. **(b,e)** Upon biological signal activation, the cytosolic form undergoes conformational change/autophosphorylation. **(c, f)** Newly formed membrane-bound protein is shown in the red circle. The depleted cytosolic population is shown in a dotted red sphere. Because the depleted population can be more readily replenished and re-activated in (c) the pathological state than (f) the healthy state, more membrane bound form in (a) than in (d) may underlie chronic activation of p52shc/Src-associated signaling cascades.