Neuron, Vol. 40, 319–330, October 9, 2003, Copyright ©2003 by Cell Press

Specific Localization and Timing in Neuronal Signal Transduction Mediated by Protein-Lipid Interactions

Review

Marc Fivaz and Tobias Meyer*
Department of Molecular Pharmacology
School of Medicine
Stanford University
318 Campus Drive
Clark Center W200
Stanford, California 94305

A large number of signaling proteins translocate from the cytosol to the plasma membrane in response to receptor and electrical stimuli. The site of translocation to the plasma membrane and the "on" and "off" rates of the translocation process are critical for defining the specificity of the signaling response. In addition to targeting mechanisms based on protein-protein interactions, signaling proteins have evolved a large repertoire of covalent lipid modifications and lipid binding protein modules that regulate reversible membrane association. The time constants of these membrane interactions range from milliseconds to several hours. Here we discuss how diversity in lipid-based membrane anchoring and targeting motifs contributes to plasticity in neuronal signaling by providing local and regional control mechanisms as well as a means to transduce and integrate signals over a broad range of different time scales.

Introduction

Neurons must convert large numbers of receptor stimuli and electrical signals into short- and long-term intracellular responses. These inputs are relayed and processed by signaling pathways that are not organized in a linear fashion but that instead display a complex network-like behavior with important lateral cross talk between different signaling modules. The question of how signaling specificity is achieved has therefore become central in the field of signaling research. Two important basic principles that have emerged in recent years are that specificity can be achieved in space by dynamic colocalization mechanisms and in time by coincidence detection mechanisms (for reviews, see Pawson and Scott, 1997; Meyer and Shen, 2000; Teruel and Meyer, 2000).

Spatial control of signaling in neurons is inherently associated with the polarized architecture of these cells and their need to integrate and propagate a vectorial flow of information. Neurons have evolved subcellular structures, such as the axon, axon hillock, dendrites, spines, cell body, and presynaptic terminals (Figure 1), that can separately transduce and integrate a vast array of local and global signal inputs. Local increases of calcium at individual spines is a well-documented example of spatially confined signaling used for selective target activation (see the review by Augustine et al. [2003] in this issue of *Neuron*).

In addition to spatially segregating signaling processes, neurons need to transduce and process information over different time scales, ranging from milliseconds in visual phototransduction (Lee et al., 2003) to days or longer in the case of persistent changes in synaptic efficacy (Malinow and Malenka, 2002). The full computational power of a neuron thus requires an impressive plasticity in the underlying signaling network to relay information over these time scales.

What are the molecular mechanisms that control localization and timing of signaling in neurons? Recent findings have led to the formulation of a dynamic model of signal transduction (the softwired signaling concept), which is based on the idea that many signaling proteins reversibly translocate from inactive sites (for example, the cytoplasm) to the target site where signaling is initiated (for reviews, see Pawson and Scott, 1997; Teruel and Meyer, 2000). Selective activation of downstream functions is then ensured by translocation and colocalization of sequentially acting signaling components. This spatially and temporally controlled assembly process relies primarily on protein modules (Sato et al., 2001; Schlessinger and Lemmon, 2003), scaffolds (Park et al., 2003), and cytoskeletal elements (Ehlers, 2002) that mediate selective protein-protein and protein-lipid interactions and control sequential docking of signaling components to target cytoskeletal structures and membranes.

An important component of these regulatory processes is the dynamic association of signaling proteins with lipid membranes. Cells have evolved an arsenal of molecular tricks that allow signaling molecules to reversibly associate with cellular membranes. These include lipid binding protein domains that mediate membrane translocation in response to receptor-stimulated lipid turnover, such as pleckstrin homology (PH), Fab1-YOP-vac1-EEA1 (FYVE), epsin amino-terminal homology (ENTH), Phox homology (PX), and C1 domains or motifs like C2 domains that respond to calcium elevation. In addition to these membrane targeting domains, a large number of signaling molecules also interact with membranes by virtue of a variety of covalent lipid modifications such as myristoylation, palmitoylation, or prenylation (Figure 2). Long thought to act as inert plasma membrane anchors, these various lipid moieties have recently been shown to regulate subcellular localization and reversible membrane association.

This review discusses how lipid targeting protein domains and covalent lipid modifications act as diverse membrane targeting devices that regulate the spatial and temporal dynamics of signaling in neurons. We will first focus on the broad structural diversity of membrane targeting motifs and then discuss how these motifs contribute to timing and localization mechanisms in signal transduction.

A Lexicon of Membrane-Interacting Motifs

Lipid binding protein modules and covalent lipid modifications are traditionally viewed as functionally very different means to target a protein to membranes. Lipid binding protein domains have evolved three-dimen-

*Correspondence: tobias1@stanford.edu

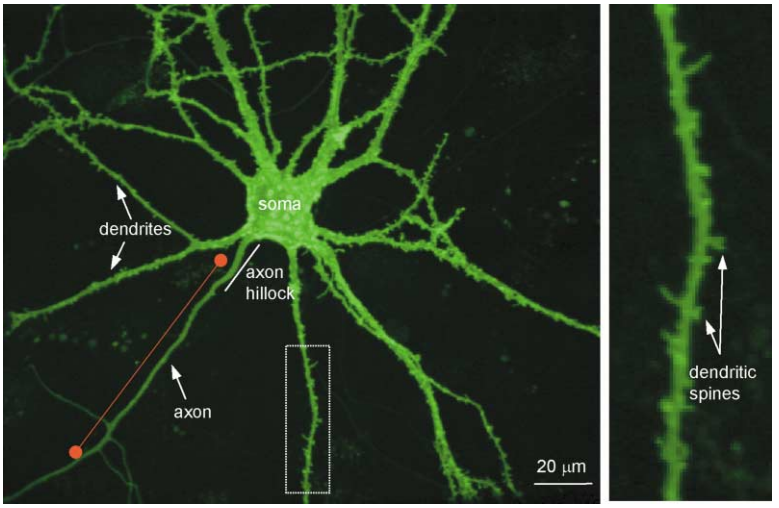

Figure 1. The Complex Membrane Architecture of Neurons

Confocal image of a hippocampal neuron expressing a CFP-tagged membrane marker. Note the difference between dendritic processes, which show many membrane protrusions or spines (better resolved in the magnified image on the right), and the axon, which has a relatively smooth membrane surface and can extend over several hundreds of microns. The red scale bar along the axon indicates the distance (70 μm) wandered by a lipid bound protein in 2 hr with a diffusion coefficient of 0.5 $\mu m^2/s$ (see text).

sional folds that specifically interact with lipids embedded in cellular membranes, whereas lipid-modified proteins interact with cellular membranes by insertion of their hydrophobic lipid moiety into the lipid bilayer. Lipid targeting protein domains can mediate highly regulated, short-lived interactions with specific lipids (see Tables 1 and 2; for reviews, see Hurley and Meyer, 2001; Wientjes and Segal, 2003), whereas lipid modifications are generally considered as long lasting, nonregulated membrane anchors. This static view of protein lipidation has changed based on recent findings showing that lipid modifications can actively contribute to the subcellular localization of signaling proteins and, in some instances, regulate reversible association with membranes. Localization and rates of translocation of lipid-modified proteins depend on the structural characteristics of the lipid moiety and on the coupling chemistry to the polypeptide. We discuss below the structural features of covalent lipid modifications that target proteins to the cytoplasmic leaflet of cellular membranes, polybasic targeting motifs that mediate low-affinity membrane interactions, and structurally defined protein domains that mediate selective protein-lipid interactions.

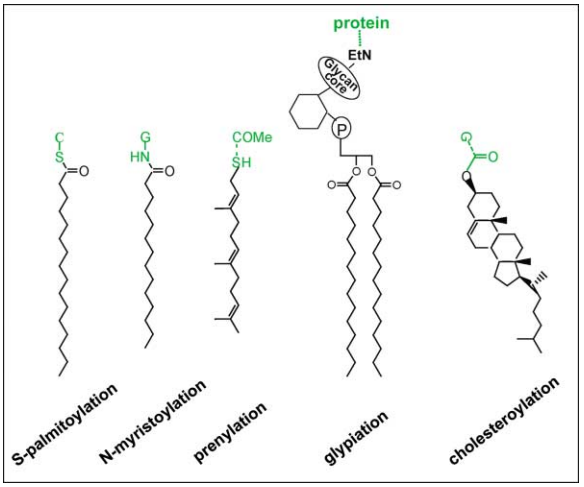

Figure 2. Covalent Lipid Modifications of Proteins

The five different classes of covalent lipid modifications. Lipid-modified signaling proteins often use a combination of these lipid anchors to regulate their association with cellular membranes (see text). Shown in green is the amino acid to which the lipid is covalently linked. Glypiation and cholesteroylation are not further discussed here, since they are found in proteins anchored to the outer leaflet of the plasma membrane.

Covalent Lipid Modifications

Palmitoylation is the most abundant and the only reversible covalent lipid modification known to date. In most palmitoylated proteins, palmitic acid, a 16 carbon fatty acid (or another long chain fatty acid), is covalently linked posttranslationally to the free thiol of a cysteine residue through a labile thioester bond (Figure 2). This thioester linkage, known as S-palmitoylation, is susceptible to hydrolysis and can be selectively cleaved by palmitoylthioesterases (Linder and Deschenes, 2003). S-palmitoylation is found in a vast range of signaling proteins. Classic examples include GPCRs, G_α s, nitric oxide synthase (NOs), the axonal growth cone associated protein GAP-43, and the postsynaptic scaffold PSD-95 as well as a number of small GTPases of the Ras superfamily, such as H-Ras (Table 2). Palmitoylation can also occur through an amide linkage with the free amine of the N-terminal amino acid (N-palmitoylation), as reported for the morphogen sonic hedgehog (Pepinsky et al., 1998) and the G protein $G_{s\alpha}$ (Kleuss and Krause, 2003) (see also Table 2). The mechanisms involved in palmitoylation are still poorly understood. The palmitoylacyltransferase (PAT) activity responsible for this modification has not been fully characterized, and no reliable predictions can be made at present as to whether a particular cysteine residue will serve as substrate for palmitoylation (Bijlmakers and Marsh, 2003).

N-myristoylation refers to the covalent addition of the 14 carbon fatty acid myristate, via an amide linkage, to the NH2 terminal of a glycine residue (Figure 2). N-myristyl transferase (NMT), the enzyme that catalyses this reaction, recognizes the consensus sequence MGXXXS/T (Towler et al., 1988). N-myristoylation usually occurs cotranslationally and is a chemically stable,

Table 1. Occurrence of Membrane Targeting Motifs in the Human Genome

Membrane Targeting Motif	Number of Motifs in the Human Genome
Lipid-based:	
Palmitoylation	?
Myristoylation	270
Prenylation (CaaX)	214
Protein-based:	
Polybasic	?
PH domains	448
ENTH domains	16
FYVE domain	66
PX domains	65
C2 domain	225
C1 domain	97

The number of lipid binding protein domains was retrieved from the PFAM database (http://www.sanger.ac.uk/Software/Pfam/). The number of predicted CaaX box-containing proteins was retrieved from the Ensembl human genome browser (http://www.ensembl.org/Homo_sapiens/). This number does not include Rab proteins prenylated by a non-CaaX motif (see text). The number of predicted human N-myristoylated proteins was retrieved from (http://mendel.imp.univie.ac.at/myristate/); see also Maurer-Stroh et al. (2002).

irreversible lipid modification (Johnson et al., 1994). N-myristoylated proteins include src-related non-receptor protein tyrosine kinases (NRPTKs), myristoylated alanine-rich C kinase substrate (MARCKS), the catalytic subunit of the cAMP-dependent protein kinase A (PKA), calcineurin (the regulatory subunit of protein phosphatase 2B), G_α s, ARFs (ADP ribosylating factors of the small GTPases superfamily), and recoverin, a calcium sensor involved in vision. The addition of myristate will not, in most cases, anchor a myristoylated protein firmly to the plasma membrane (McLaughlin and Aderem, 1995). Additional elements, such as palmitoylation or a polybasic domain, are required for efficient association with membranes (Table 2). Posttranslational N-myristoylation has also been observed as a result of proteolytic cleavage and exposure of an internal glycine residue (Zha et al., 2000).

Prenylation consists of the covalent attachment of a C15 (farnesyl) or C20 (geranylgeranyl) isoprenoid moiety to a C-terminal cysteine residue, via thioether linkage (Figure 2). Prenylation of a C-terminal cysteine residue occurs in the context of prenylation motifs, which fall into two distinct classes: CaaX-containing motifs (Fu and Casey, 1999), which are found in a variety of different signaling molecules; and non-CaaX motifs (Pereira-Leal et al., 2001), which are specific to Rab proteins. CaaX and non-CaaX prenylation motifs employ a different set of prenylation enzymes. Farnesyltransferase (FT) and geranylgeranyltransferase type I (GGTI) recognize the tetrapeptide CaaX, where C is the cysteine to be isoprenylated, a is an aliphatic amino acid, and X is any amino acid (Fu and Casey, 1999). Rab geranylgeranyl transferase type II (GGTII), in contrast, acts on prenylation motifs that frequently consist of two nearby cysteine residues, both of which get geranylgeranylated (Pereira-Leal et al., 2001). For CaaX-containing proteins, specificity for FT and GGTI is determined by the nature of X. When X is a methionine or a serine, the protein gets farnesylated, as in the case of Ras proteins. When X is a leucine, the protein becomes geranylgeranylated, as in the case of Rho proteins (Fu and Casey, 1999; Pereira-Leal et al., 2001). Once prenylated, CaaX proteins are further processed by endoproteolitic removal of –aaX (by the Rce1 endoprotease [Bergo et al., 2002]) and carboxymethylation of the C-terminal cysteine residue (Bergo et al., 2000) (via an isoprenylcysteine carboxyl methyltransferase [ICMT]). These two processing steps are believed to be important for correct membrane targeting of CaaX-containing proteins. In addition to Ras-like small GTPases, prenylation is found in a variety of other proteins, including γ subunits of trimeric G proteins, retinal cGMP phosphodiesterase, G protein-coupled receptor kinases, and nuclear lamins.

Polybasic Domains

Polybasic domains refer to a relatively short cluster of basic amino acids ($<$30 aa) that mediate nonspecific binding to acidic lipids via electrostatic interactions. A polybasic domain leads to preferential targeting to the plasma membrane (relative to other subcellular compartments), presumably because the plasma membrane is enriched in acidic phospholipids. Polybasic domains usually act synergistically with other membrane targeting motifs, such as myristoylation and prenylation, to promote efficient targeting to the plasma membrane (Table 2). However, relatively long polybasic domains, such as those found in the small GTPases Rit or Rin (Lee et al., 1996), generate sufficient electrostatic interactions to drive membrane translocation on their own. Such interactions of polybasic domains with membranes are of low affinity and are rapidly reversible, a property which is central to a number of switch-like mechanisms that modulate reversible protein-membrane interactions.

Phosphoinositides Binding Modules

In addition to the relatively long-lasting membrane interactions mediated by some covalent lipid modifications, cells have also evolved a repertoire of low-affinity lipid interaction modules that regulate transient association with cellular membranes. Interestingly, most of these lipid binding domains mediate interactions with different species of phosphoinositide lipids. Three of the phosphoinositide lipids, $PI(4,5)P_2$, $PI(3,4,5)P_3$, and PI(3)P, stand out as important protein binding partners (Hurley and Meyer, 2001; Lemmon, 2003).

First, many of the pleckstrin homology (PH), Four-point-one-ezrin-radixin-moesin (FERM), and ENTH domains have been shown to bind selectively to $PI(4,5)P_2$. A number of proteins, including transporters, channels, and cytoskeletal regulators (such as gelsolin or cofilin), also have been shown to interact with $PI(4,5)P_2$ by other mechanisms that do not involve the aforementioned $PI(4,5)P_2$ binding modules. In addition to its role as a ubiquitous membrane anchor, $PI(4,5)P_2$ also functions as a second messenger, involved in regulation of the cytoskeleton (Raucher et al., 2000), channels and transporters (Hilgemann et al., 2001), and membrane trafficking (De Matteis et al., 2002; Slepnev and De Camilli, 2000). The diverse structural motifs for $PI(4,5)P_2$ binding

Table 2. Membrane-Targeting Motifs

Membrane-Targeting Motif	Proteins	Motif Sequence	Localization (Relative Enrichment)	Lipid Specificity
Lipid-Based				
S-palmitoylation	GAP-43	N_{term}- MLCCMRRTKQV	PM, axone	DRMs
	PSD-95	N_{term}- MDCLCIVTTKKY	PM, dendritc spines	DRMs
	ABP-L	N_{term}- MRGWLRRNLALCLQRPLP	PM, dendritc spines	n.d.
N-palmitoylation/S-palmitoylation	$G_{s\alpha}$	N_{term}- N-GCLGNSKTE	PM	DRMs
Myristoylation/palmitoylation	Lyn	N_{term}- MGCIKSKGKD	PM, Golgi	DRMs
	$G_{i\alpha1}$	N_{term}- MGCTLSAEDK	PM	DRMs
	Fyn	N_{term}- MGCVQCKDKEATKLTE	PM, Golgi	DRMs
N-palmitoylation/cholesteroylation	hedgehog	N_{term}-N-CGPGP....G-C_{term}	PM (outer leaflet)	DRMs
Myristoylation + polybasic	src	N_{term}- MGSNKSKPKDASQRRSLEPD	PM	DRMs
	MARKCS	N_{term}- MGAFSKTAAK ...*(polybasic ed)*...	PM	PI(4,5)P2
Farnesylation/palmitoylation	N-Ras	LNSSDDGTQGCMGLPCVVM-C_{term}	PM, Golgi	DRMs
	H-Ras	LNPPDESGPGCMSCKCVLS -C_{term}	PM, Golgi	DRMs
	paralemmin	DMKKHRCKCCSIM-C_{term}	PM, axone	n.d.
Farnesylation + polybasic	K-Ras	KMSKDGKKKKKSKTKCVIM-C_{term}	PM	n.d.
Geranylgeranylation	Rab8	GVKITPDQQKRSSFFRCVLL-C_{term}	LE	n.d.
Dual geranylgeranylation	Rab5a	GGGVDLTEPTQPTRNQCCSN-C_{term}	EE	n.d.
	Rab14	EPIKLDKNDRAKASAESCSC-C_{term}	EE	n.d.
Geranylgeranylation/palmitoylation	Rap2b	NTAAQSNGDEGCCSACVIL-C_{term}	PM	n.d.
Geranylgeranylation + polybasic	Rap1a	NRKTPVDKKKPKKKSCLLL-C_{term}	PM	n.d.
GPI-anchor	PrP	not shown	PM (outer leaflet)	DRMs
Protein-Based				
Polybasic	Rit	KNSVWKRLKSPFRKKKDSVT-C_{term}	PM	acidic PLs
PH domains	PLC-δ1	not shown	PM, cytosol	PI(4,5)P2
	Akt	not shown	PM, cytosol	3' PIs
FERM	PTPL1	not shown	PM	PI(4,5)P2
ENTH domain	epsin	not shown	PM (CCPs)	PI(4,5)P2
C2 domain	cPKC	not shown	PM	PS
	$cPLA_2$	not shown	ER, Golgi, PM	PC
C1 domain	cPKC	not shown	PM	PS
FYVE domain	EEA1, Hrs	not shown	EE	PI(3)P
PX domain	NADPH oxidase	not shown	PM	PI(4,5)P2
	Sorting nexins	not shown	EE, LE	PI(3)P

Red C, S-palmitoylated cys; pink C, putative palmitoylated cys; red N, N-palmitoylated amino-terminal aa; green G, N-myristoylated glycine; purple G, cholesterol-modified glycine. CaaX motifs are shown in orange. Basic amino acids are in blue. PM, plasma membrane; ER, endoplasmic reticulum; EE, early endosomes; LE, late endosomes; CCP, clathrin-coated pits. All motifs target the inner leaflet of cellular membranes, except GPI-anchors and cholesterol modifications. Please note that this table does not appear in color in the online version due to technical limitations. Please see the print version or the downloadable PDF file (available online) for the full color version of this table.

found in a large number of neuronal proteins raise many questions about its role in neurons.

Second, $PI(3,4,5)P_3$ lipids have emerged as one of the most important second messengers that control local and global signaling in cells, membrane trafficking, and an array of other key cellular processes. A subclass of PH domains, found in the Akt, Pdk1, and Btk kinases (Ferguson et al., 2000; Isakoff et al., 1998) as well as in the exchange factors ARNO and Grp1 (Macia et al., 2000; Venkateswarlu et al., 1998), are known to trigger the translocation of signaling proteins in response to $PI(4,5)P_3$ production. These specific interactions of $PI(3,4,5)P_3$ with PH domains turned out to have structurally little in common with the interactions of $PI(4,5)P_2$ with other classes of PH domains (Baraldi et al., 1999). $PI(3,4,5)P_3$ has also been reported to bind to a number of other signaling proteins that do not have PH domains, through a mechanism that presumably involves the high charge density of this lipid.

The third major group of phosphatidylinositol-phosphate interactions involves PI(3)P, a lipid that regulates endosomal trafficking. While there is no PH domain known to interact with this lipid, the Zn^{2+}-containing Fab1-YOTP-Vac1-EEAI (FYVE) domain has been shown to selectively bind to PI(3)P. FYVE domains usually function in tandem or together with other signaling domains (Stenmark et al., 2002). Different domains that bind PI(3)P (and also dually phosphoryated PI lipids) are PX domains (Wientjes and Segal, 2003). Since both these domains are probably more important in vesicle trafficking than in cell signaling, we will not discuss them in this review.

C1 and C2 Domains: Diacylglycerol and Ca^{2+}-Driven Membrane Translocation

Ca^{2+}-regulated C2 domains constitute an interesting class of protein translocation modules that can relocalize proteins in response to Ca^{2+} signals. There are at least two different types of Ca^{2+}-sensitive C2 domains that mediate interactions with the plasma membrane (for example, conventional protein kinase C [cPKC]) and with internal membranes (for example, $cPLA_2$), respectively. The C2 domain of cPKC preferentially binds acidic lipids in the presence of Ca^{2+} (Bolsover et al., 2003); the one from $cPLA_2$ binds to neutral membranes, which are rich in phosphatidylcholine (PC) (Perisic et al., 1999).

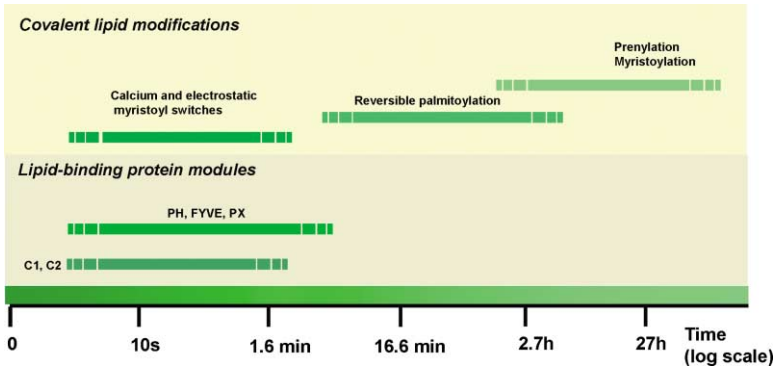

Figure 3. Putative Time Constants for Membrane Association of Lipid-Modified Proteins and Lipid Binding Protein Modules

Approximate time scales of interaction between lipid membranes and different lipid binding protein modules and lipid-modified proteins. Note that for some prenylated proteins (i.e., small GTPases of the Rab family), turnover can be accelerated by GDP dissociation inhibitor proteins (GDIs). The time scales shown here are rough estimates.

Presumably, the higher acidic lipid content in the inner leaflet of the plasma membrane in comparison to a more neutral lipid composition of the cytosolic leaflet of internal membranes could explain the differences in targeting. In addition to these two types of membrane interactions, other C2 domains can promote Ca^{2+}-insensitive membrane interactions.

Unlike C2 domains, C1 domains are recruited to membranes by in situ generation of diacylglycerol (Cho, 2001). While less is understood about the regulated generation of diacylglycerol in internal membranes, a predominant pathway leading to the production of DAG at the plasma membrane involves phospholipase C-mediated hydrolysis of $PI(4,5)P_2$ (Taylor, 2002), suggesting that plasma membrane diacylglycerol signals are coupled to the production of InsP3 and intracellular Ca^{2+} release. Like the C2 domains, C1 domains have structural surfaces with positively charged amino acids, suggesting that they recognize diacylglycerol in combination with acidic lipids present in the inner leaflet of the plasma membrane (Cho, 2001).

Reversible Membrane Targeting and Timing in Signal Transduction

In order to illustrate how the different membrane translocation mechanisms discussed above can confer specific timing properties in signaling, we describe here three examples where the time constant of membrane association is adapted to a particular physiological context. (1) Reversible S-palmitoylation at the synapse, (2) the myristoyl switch and the regulation of light adaptation in vision, and (3) generation of a temporal coincidence detector in PKC by sequential engagement of multiple lipid interaction modules. Figure 3 gives an overview of the approximate time constants of different membrane interactions conferred by membrane interaction motifs.

Reversible S-Palmitoylation and Synaptic Plasticity

One conceptually simple way to regulate membrane translocation of a lipid-modified protein is to control the rates at which the protein gets lipidated and delipidated. Such a reversible lipidation process is found in proteins that are modified by S-palmitoylation.

Palmitate turnover has been observed in several proteins, including neuronal nitric oxide synthase (nNOs), GAP-43, H-Ras, and recently PSD-95 (El-Husseini Ael and Bredt, 2002), with time constants in the basal rate ranging from 20 min for H-Ras (Magee et al., 1987) to 20–90 min for G_α s (Linder and Deschenes, 2003) and 2 hr for PSD-95 (El-Husseini Ael and Bredt, 2002). Most interestingly, rates of palmitate cycling can be regulated by receptor activity. For instance, palmitate turnover on G_α s increases 10-fold upon receptor stimulation (Wedegaertner and Bourne, 1994). The functional consequences of this agonist-dependent increase in palmitoylation turnover are not completely understood, primarily because an increase in palmitoylation turnover often involves several components of a signaling pathway and causes pleiotropic effects on downstream signaling (see for instance the complex case of palmitoylation turnover and GPCR signaling reviewed in Qanbar and Bouvier, 2003). Nevertheless, receptor-triggered increase in palmitoylation turnover has been associated in the case of G_α s and endothelial nitric oxide synthase (eNOS) with a net decrease in signaling activity (Robinson et al., 1995; Tu et al., 1997).

A conceptually similar role of palmitate cycling has recently been reported in both postsynaptic and presynaptic manifestations of synaptic plasticity. El-Husseini and coworkers have shown that palmitate cycling on PSD-95 in hippocampal neurons is regulated by synaptic activity (El-Husseini Ael et al., 2002). PSD-95 is a scaffolding protein located at the tip of dendritic spines in the postsynaptic density (PSD) of glutamatergic excitatory synapses. PSD localization of PSD-95 requires palmitoylation of two nearby cysteine residues, at the N terminus of the protein. PSD-95 is a multifunctional protein that interacts indirectly with AMPA-Rs, via a protein called stargazin (see review by Bredt and Nicoll (2003) in this issue of *Neuron*). PSD-95 controls, among other things, clustering and levels of AMPA-Rs at the synapse. The authors found that blockage of synaptic activity using glutamate receptor antagonists decreases palmitate turnover on PSD-95 by a factor of two. In addition, inhibition of palmitoylacyltransferase (PAT) activity with 2-bromo-palmitate (which leads to depalmitoylation of PSD-95 due to ongoing palmitate turnover) disperses PSD-95/AMPA-R clusters at the synapse and decreases AMPA-R-mediated miniature postsynaptic currents (mEPSCs), indicating that palmitate turnover at the synapse is required for AMPA-R clustering and synaptic functions. Furthermore, acute and prolonged synaptic activity causes endocytosis of AMPA-Rs, presumably via increased depalmitoylation (and diffusion

out of the PSD) of PSD-95. Taken together, these data point to a novel role of palmitate turnover in regulating activity-dependent changes in synaptic efficacy. In this particular form of synaptic plasticity, activity-dependent synaptic remodeling occurs at a rate dictated by the half-life of palmitate turnover at the synapse, which is in the order of hours, at least for PSD-95.

Modulation of palmitoylation turnover has also been shown to regulate the activity of growth-associated proteins (GAPs) in axonal growth cones (Patterson and Skene, 1999). In the developing nervous system, a critical functional transition occurs in the axonal growth cone as it reaches its postsynaptic target and matures into a functional synapse. The molecular machinery that controls growth must be replaced by a set of proteins that regulate presynaptic functions. The activity of GAPs, which regulate neurite outgrowth and axon pathfinding, needs therefore to be shut down upon synaptic contact. GAP-43, one of the best-characterized GAPs, is dually palmitoylated, a modification that targets it to the axon growth cone and that is essential for activity. Patterson and coworkers have found that GAP-43 and other palmitoylated growth cone proteins undergo depalmitoylation upon establishment of synaptic contacts. This developmental switch in S-palmitoylation has been proposed to disengage the signaling machinery that controls axonal growth, a prerequisite for the establishment of synaptic circuitry.

N-Myristoylation and Molecular Switches

Neuronal calcium sensors (NCS) constitute a growing family of EF hand Ca^{2+} binding proteins that are covalently modified by N-myristoylation (Braunewell and Gundelfinger, 1999). A large number of NCS proteins reversibly associate with membranes by an elegant mechanism known as the calcium myristoyl switch, first described in recoverin, an abundant retinal protein involved in the regulation of visual phototransduction (Ames et al., 1996). This mechanism is based on a calcium-triggered conformational change that extrudes the acyl modification from a buried hydrophobic pocket, enabling the protein to insert into a membrane (Ames et al., 1997). Membrane association and dissociation rates of recoverin (and presumably other NCSs) are in the order of seconds, (Beven et al., 2001). Such fast response kinetics of recoverin are likely to play an important role in the modulation of the phototransduction cascade and mechanisms of light adaptation.

Other studies in nonneuronal cells have led to the identification of mechanistically related but distinct myristoyl switches that operate with different triggers, such as GTP, phosphorylation, or Ca^{2+}/CaM. A GTP myristoyl switch controls reversible membrane binding of the Ras-related small GTPase ARF1 in a GTP-dependent manner (Goldberg, 1998). Nucleotide-dependent membrane association and dissociation cycles of ARF1 play a pivotal role in the dynamic assembly of coatomer coats on Golgi membranes, a process that regulates vesicular trafficking between Golgi cisternae (Spang, 2002). Phosphorylation- and Ca^{2+}/CaM-dependent myristoyl switches have been shown to operate on the same protein, MARKCS, by a mechanism referred to as the electrostatic myristoyl switch, which depends on electrostatic interactions with membranes rather than a conformational change (McLaughlin and Aderem, 1995). As discussed earlier, N-myristoylation does not usually provide sufficient hydrophobicity to drive efficient insertion in a lipid bilayer. Instead, the 14 carbon fatty acid often acts in combination with a stretch of polybasic amino acids that confers increased affinity for negatively charged phospholipids. In the case of MARKCS, this polybasic domain, known as the effector domain (ED), contains a PKC phosphorylation site and binds Ca^{2+}/CaM with nanomolar affinity (Arbuzova et al., 1997). Phosphorylation and Ca^{2+}/CaM binding to the ED are mutually exclusive events, but both lead independently to the release of MARKCS from membranes, presumably by disrupting the electrostatic interactions of MARKCS ED with the lipid bilayer (Arbuzova et al., 1997). MARCKS association with membranes is thus regulated by an electrostactic myristoyl switch, which is under the control of both PKC and Ca^{2+}/CaM signaling pathways. Given the relatively large number of proteins that are targeted to membranes via a polybasic domain, one might reasonably predict that modulation of membrane translocation by an electrostatic switch may turn out to be a common mechanism. In fact, it has been reported that the PH domain of the GEF ARNO is under the control of an electrostatic switch, triggered by PKC phosphorylation (Santy et al., 1999).

While most proteins are myristoylated cotranslationally, posttranslational N-myristoylation can occur upon exposure of an internal glycine residue following endoproteolytic cleavage. It has now been established that posttranslational N-myristoylation can act as a molecular switch that irreversibly engages the cell into a death pathway (Zha et al., 2000). Stimulation of death receptors leads to activation of a proteolytic cascade that eventually results in the cleavage of the proapoptotic factor Bid. Zha and coworkers have shown that Bid gets N-myristoylated upon proteolytic cleavage, a posttranslational modification that targets Bid to mitochondria, enhancing Bid-induced release of cytochrome c and death.

Sequential Engagement of C2 and C1 Domains in PKC Activation

An interesting example for the use of multiple lipid binding modules to build a coincidence detector is found in conventional protein kinase C isoforms, which contain two C1 domains and one C2 domain. The activity of this enzyme is turned on by a dual Ca^{2+} and diacylglycerol signal (Oancea and Meyer, 1998). Different cellular and biochemical studies have shown that the initial plasma membrane translocation of all three conventional isoforms (α, β, and γ) tightly correlates with an increase in Ca^{2+} concentration, suggesting that the first trigger for PKC activation is a Ca^{2+}-mediated interaction between the C2 domain and the inner leaflet of the plasma membrane. This leading role of the C2 domain is further supported by the evidence that GFP-conjugated C2 domains alone exhibit nearly the same receptor-triggered plasma membrane translocation kinetics as the full-length protein.

Interestingly, diacylglycerol or phorbol ester can also effectively trigger the translocation of GFP-conjugated

C1 domains from PKC to the plasma membrane. The phorbol ester-triggered translocation of the full enzyme is much weaker and delayed, suggesting that, in inactive PKC, the C1 domain is less accessible than the C2 domain. This led to a model of a sequential activation mechanism of conventional protein kinase C that begins with the Ca^{2+}-driven translocation of PKC to the plasma membrane. As a further priming step, the membrane-localized enzyme then occasionally opens up by a weak interaction between the positively charged autoinhibitory region at the N terminus of cPKCs and the plasma membrane. This reversible opening and loss of autoinhibition is likely responsible for the observed partial activity of the enzyme in the presence of Ca^{2+} and in the absence of diacylglycerol. If diacylglycerol is then present during this second short opening step, the C1 domains can bind to diacylglycerol, and the catalytic domain is freed from the self-inhibition by the regulatory domain. This suggests that the C1 and C2 domains as well as the autoinhibitory N-terminal peptide of PKC form an intricate coincidence detector that is based on four separate plasma membrane interactions (two C1s, one C2, and one polybasic) and which enables cells to selectively respond to Ca^{2+} and DAG signals. Similar combinations of lipid binding modules and other regulatory control modules are found in many signaling proteins, suggesting that modular lipid and protein interactions provide a rich opportunity for cells to build molecular machines with desired coincidence detection, integration, and delay properties.

Local and Regional Signaling Mechanisms Resulting from Lipid-Protein Interactions

In addition to the importance of timing, the second key aspect of membrane targeting of signaling proteins is the spatial selectivity of the translocation process. In this last section, we focus on four issues related to the control of local (submicrons to microns) and regional (tens of microns) signaling processes in neurons by membrane targeting motifs. (1) The establishment and maintenance of a polarized distribution of signaling proteins against lateral diffusion. (2) Local activation of signaling proteins by spatially confined production of lipid second messengers. (3) The role of lipid microdomains or rafts in spatial segregation of signaling complexes. (4) Targeting of signaling activity to intracellular membranes.

Neuronal Polarity, Transport, and Diffusion of Lipid Bound Signaling Proteins

The vectorial flow of information that propagates from postsynaptic membranes to presynaptic terminals relies on the polarization of neurons into structurally and functionally defined dendritic and axonal membrane regions with different protein and lipid compositions. The establishment and maintenance of neuronal polarity is a complex process that depends on protein/lipid sorting mechanisms (see the review by Horton and Ehlers [2003] in this issue of *Neuron* for a detailed description of the mechanisms involved in neuronal polarity). By analogy with sorting mechanisms that control polarity in epithelial cells, one current model proposes that axonal and dendritic proteins are selectively incorporated into two different classes of TGN-derived transport vesicles that traffic to either axons or dendrites (Dotti and Simons, 1990). Alternatively, neuronal polarity could be achieved by specific retention rather than specific sorting of proteins to the axonal or somatodendritic membranes (Jareb and Banker, 1998). Both mechanisms can operate in the same cell for different cargos, as recently shown by Banker and coworkers (Sampo et al., 2003).

Recently, S-palmitoylation has been identified as an axonal sorting signal for some (but not all) peripheral membrane proteins. For example, dual palmitoylation on two adjacent N-terminal residues targets GAP-43 to the axon (El-Husseini Ael et al., 2001). Preferential axonal localization of palmitoylated proteins involves a mechanism that depends most likely on selective sorting of the cargo in TGN-derived vesicles destined for the axonal membrane. Based on the observation that lipid-modified proteins do not usually have specific endocytic motifs or, by definition, ectopic protein domains that could regulate specific membrane retention, the axonal distribution of GAP-43 is, indeed, unlikely to be mediated by selective endocytosis or active membrane retention mechanisms. Therefore, the critical issue for the establishment/maintenance of the polarized axonal distribution of lipid-modified proteins (and possibly a large number of other proteins) is the competition between an active transport/targeting mechanism and a passive lateral membrane diffusion process. Note that this problem is also relevant for local protein distributions within axons, dendrites, or the cell soma.

All diffusion measurements of membrane-anchored proteins (i.e., lipid-modified and lipid binding modules) have led to diffusion coefficients that are in fairly close agreement, in the order of $\sim$0.5 $\mu m^2/s$ (Niv et al., 2002; Oancea et al., 1998). As for all diffusion processes, locally membrane-delivered proteins then spread over distances that increase with the square root of time. Thus, in 1 s, a membrane bound protein diffuses about 1 μm from its origin; in 1 min, 6 μm; and in 1 hr, 50 μm; which is relatively little compared, for instance, to the length of an axon, which often extends above 1 mm. Figure 1 provides an idea of how such dimensions relate to the dendritic and axonal geometry of neurons. The extended dimensions of neurons can therefore readily account for the maintenance of a polarized distribution as long as the protein gets delivered sufficiently rapidly and selectively to either dendrites or axons.

While these considerations show that polarized axonal versus somatodendritic distribution of membrane bound proteins may not be limited by diffusion, the same is not true for targeting to more local sites. In order for a membrane-associated signaling protein to be targeted to a confined region, the protein has to be delivered and removed from the site within the time it takes for the protein to diffuse away from the desired region. Figure 4 shows a graph of the diffusion range of a locally delivered protein. This graph shows that the degree of microlocalization of a membrane bound signaling protein is closely linked to its half-life at the membrane. The same concept can be extended to the degree by which a locally generated signaling activity remains local (for example, a local GDP to GTP exchange can create a local source of active small GTPases). In the case where a persistently membrane-associated protein is locally

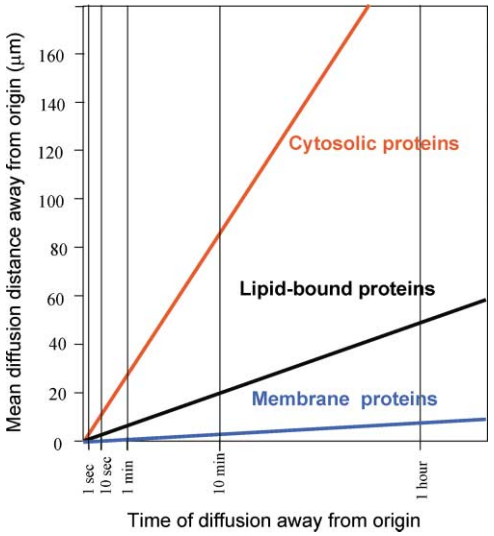

Figure 4. Approximate Mean Diffusion Distances of a Lipid Bound Protein in the Plane of the Plasma Membrane

The approximate diffusion of transmembrane and cytosolic proteins are shown for comparison. The term "lipid bound" refers in this context to both lipid-modified and lipid binding protein modules. A square root time axis was used to linearize the diffusion plot. The lines shown here are derived from numbers discussed in the text.

activated, the degree of microlocalization of signaling depends on the half-life of inactivation rather than the half-life of membrane association.

Even though all lipid-anchored proteins (in the inner leaflet of the bilayer) have similar lateral diffusion coefficients, it also has to be considered that membrane-associated proteins can bind in addition to lipids to other peripheral signaling proteins, cytoskeletal elements, or membrane microdomains, thereby reducing the apparent diffusion coefficient and the spread of a locally generated signal. Further studies are needed to better understand the importance of mechanisms that reduce lateral membrane diffusion in neurons.

Spatially Restricted Signaling Controlled by Local Production of Lipid Second Messengers

Local signaling by lipid second messengers provides a good example to further illustrate some of the diffusion concepts described above. Local production of PIs with relatively short half-lives has proven to be a powerful mechanism to spatially segregate signaling pathways in different cellular contexts. The involvement of PIs and, in particular, PI(3,4,5)P_3 lipids in signaling pathways that determine cell polarity and asymmetry during development hints at an important role of PI metabolism in mediating local or regional responses. Recent work on cultured neurons by Song-Hai Shi and coworkers (Shi et al., 2003) now shows that local PI(3,4,5)P_3 signaling at the tip of an axonal growth cone plays a pivotal role at an early step in the establishment of neuronal polarity. The authors have found that PI3K-dependent synthesis of PI(3,4,5)P_3 at an axonal growth cone is required for axon specification and acts through recruitment of a signaling complex composed of mPar3, mPar6 (two mammalian homologs of *C. elegans* genes involved in anterior-posterior polarity), and atypical protein kinase C. Confined PI(3,4,5)P_3 signaling in the axonal growth cone requires a turnover of PI(3,4,5)P_3 of ~1 min [given the diffusion coefficient of PI(3,4,5)P_3 and the size (~10 μm) of a growth cone]. Persistent (over hours) local PI(3,4,5)P_3 signaling is however reported in this study, strongly suggesting that local PI(3,4,5)P_3 signaling is maintained by rapid cycles of PI(3,4,5)P_3 synthesis and degradation. Whether this balance in PI(3,4,5)P_3 signaling is determined by the relative activity of PI(3) kinase (PI3K) and the PI(3,4,5)P_3 phosphatase PTEN, as shown in PIP_3-mediated signaling at the leading edge of a chemotaxing cell (Funamoto et al., 2002), remains to be established.

In addition to the critical regulatory control mechanisms based on PI(3,4,5)P_3 signals, signaling proteins also utilize a different phosphoinositide anchor, PI(4,5)P_2. The large number of proteins that interact with this lipid and the many regulatory control mechanisms that up- and down-regulate local PI(4,5)P_2 concentration suggest that this lipid may be even more critical for cell function than PI(3,4,5)P_3 lipids. Key regulatory roles of PI(4,5)P_2 signals are related to the local remodeling of the actin cytoskeleton, to the regulation of channels and transporters, as well as to the control of membrane trafficking. While most of these roles of PIP_2 have been investigated in nonneuronal cells, it is thought that PIP_2 is a near-universal second messenger that can generate localized signals with spatial dimensions between nanometers and micrometers.

A recent study of PIP_2 signaling in neurons using GFP-conjugated PH domains showed that electrical stimuli can release PI(4,5)P_2 binding PH domains from the plasma membrane by a Ca^{2+}-dependent mechanism (Micheva et al., 2001). Furthermore, NMDA receptor activation as well as a regulatory contribution from nitric oxide synthase (NOs) seems to be critical for this down-regulation of PIP_2 signaling (Micheva et al., 2003). In contrast to these two phosphoinositide second messengers, much less is currently known about where and when diacylgycerol is produced in neurons or whether local Ca^{2+} signals indeed drive local C2 domain translocations, as could be expected from studies in other cell types.

Compartmentalization of Signaling Pathways in Submicron Membrane Microdomains

A number of lipids with relatively long and saturated acyl chains [i.e., palmitoylation or PI(4,5)P_2] have been shown to confer affinity for liquid-ordered, cholesterol-rich, detergent-resistant membrane domains both in vivo and in vitro (Brown and London, 2000). The size of these membrane domains, or rafts, has been estimated in independent studies to be in the tens to hundreds of nanometers scale (Pralle et al., 2000; Prior et al., 2003; Varma and Mayor, 1998). Importantly, the size and composition of rafts can be dynamically altered by the "signaling status" of membrane-associated signaling complexes, suggesting that rafts may serve as regulated submicron platforms that spacially organize signaling pathways at the plasma membrane. Such an activity-dependent partitioning in and out of rafts has recently been observed for H-Ras at the plasma membrane and is likely to be functionally important (Prior et al., 2001).

From a functional perspective, local concentration of a subclass of signaling proteins in lipid microdomains offers a unique mechanism to segregate signaling processes into spatially distinct (raft and non-raft) membrane domains. This differential localization of membrane-anchored signaling proteins may enable a form of signaling selectivity based on membrane partitioning at the submicron level. Importantly, transient association of signaling proteins with rafts may be sufficient to promote a specific set of protein-protein interactions required to trigger a particular signaling event.

Although lipid rafts have now been implicated in numerous signaling pathways in nonneuronal cells (see Simons and Toomre, 2000, for a review), much remains to be done to determine the protein/lipid composition of these domains and, most importantly, to determine to what extent they are functionally involved in the spatial regulation of signaling pathways. Particularly in neurons, the potential roles of rafts and other microdomains in controlling the localization and timing of signaling processes have still to be addressed.

Lipid Modifications and Intracellular Signaling

Prenylation in the context of Ras signaling is perhaps one of the most striking examples of how lipid-based membrane-targeting motifs can differentially regulate compartmentalization of signaling proteins. Two highly homologous Ras isoforms, H-Ras and K-Ras (often referred to as K-Ras4B), differ essentially in their C-terminal hypervariable regions, which contain their membrane-anchoring motif (Table 2). Although both isoforms are farnesylated through a common CaaX signal, H-Ras is further palmitoyled on two cysteine residues, while K-Ras contains a polybasic domain. These accessory membrane-targeting signals are required for efficient plasma membrane localization of both isoforms. The mechanisms by which H-Ras and K-Ras are targeted to the plasma membrane are however fundamentally different.

In an elegant series of experiments, Choy and coworkers have shown that the CaaX prenylation motif targets proteins to the endomembrane system (ER and Golgi) rather than to the plasma membrane (Choy et al., 1999). Targeting to the ER allows further processing of the prenyl modification (proteolytic cleavage and carboxymethylation) by ER localized enzymes. From there, H-Ras associates with the cytoplasmic leaflet of the Golgi, through its palmitoylated cysteine residues, and is transported to the plasma membrane by vesicular traffic. K-Ras, in contrast, is routed to the plasma membrane by a yet undefined, presumably nonvesicular mechanism, possibly through interaction with microtubules (Chen et al., 2000). These two different sorting mechanisms lead to different subcellular localization of the two Ras isoforms. K-Ras is almost exclusively found at the plasma membrane, whereas a significant fraction of H-Ras is associated with the Golgi apparatus at steady state. This intracellular localization of H-Ras has unexpected consequences on the regulation of H-Ras signaling.

Receptor-triggered activation of Ras (and other signaling molecules) was traditionally envisioned as a process that takes place exclusively at the plasma membrane. Exciting work by Bivona and coworkers (Bivona et al., 2003) now shows that receptor stimulation also activates a pool of H-Ras localized in the Golgi apparatus. Activation of Golgi-localized H-Ras occurs through a calcium-dependent recruitment of RasGRP1 (a Ras GEF) to Golgi membranes. Interestingly, calcium also translocates CAPRI, a Ras GAP, to the plasma membrane, thereby inactivating plasma membrane localized H-Ras. These data have led to a challenging model where intracellular versus plasma membrane signaling of H-Ras is determined by the relative levels of RasGRP1 and CAPRI on Golgi and plasma membranes, respectively, which may further be controlled by the duration and amplitude of the calcium signal. Whether such a regulatory mechanism is observed in neurons remains to be shown. This could potentially have marked implications in the context of the Ca^{2+}-dependent Ras/MAPK pathway that relays synaptic inputs to the regulation of gene transcription in the nucleus (Dolmetsch, 2003). The complex Ca^{2+} signals generated by different synaptic and receptor stimuli may therefore be mirrored by selective regulation of either plasma membrane- or Golgi-localized H-Ras activity. As a possible mechanism, activation of H-Ras on the Golgi could facilitate transmission of the signal to the nucleus, due to the close proximity of these two compartments.

Conclusions

The central role of protein-membrane interactions in signal transduction raises a number of important issues. In neurons, where the relative surface area to volume is much higher than in other cells, membrane-localized signaling processes are particularly important. One main aspect of membrane interactions based on covalent lipid modifications and lipid binding interactions is the relatively large diffusion coefficient of lipid bound proteins. The diffusion coefficient of transmembrane proteins, which are often attached to cytoskeletal and other membrane structures, is one or more orders of magnitude slower than that of lipid-anchored proteins. The fast lateral diffusion enables peripheral signaling proteins to be rapidly spread in two dimensions.

An obvious advantage of lipid bound signaling proteins is therefore to spatially propagate a locally generated signal and to integrate signals over a larger surface region. Furthermore, as best illustrated in the highly amplified visual phototransduction cascade, the rapid spreading of a locally generated signal can contribute to amplification processes, since a large area with many target proteins can be rapidly screened by a fast-diffusing signaling protein.

A second advantage of membrane-localized signaling is conferred by the reversible nature of membrane interactions. Membrane targeting offers the possibility to transiently assemble sequentially acting signaling proteins by reversibly recruiting different components of a signaling pathway. This can even occur at the level of an individual protein, where different domains can be recruited to the plasma membrane by sequential signaling events as was discussed above for PKC. The obvious advantages for reversible membrane recruitment are the generation of better coincidence detection mechanisms (or "AND gates") as well as the suppression of basal

activity, since signaling proteins can be kept away from each other before translocation. By limiting the reversible recruitment to a particular site, colocalization is a powerful mechanism to also confine a signaling pathway and the resulting cellular responses to a particular cellular region. As discussed above, the large number of different lipid modifications and lipid interaction domains provides an array of different mechanisms to localize signaling proteins to different membrane regions.

Given the large number of signaling processes that occur at the plasma membrane, the question has been repeatedly raised why this membrane plays such a central role in signal transduction. In addition to the obvious fact that most receptors are localized at the plasma membrane, it is widely believed that signaling processes in two dimensions are more efficient than those in three dimensions. The translocation from the cytosol to the plasma membrane confines a protein to a region of a few nanometers near the plasma membrane and leads therefore to a large increase in its concentration (about 1000-fold, as reviewed in McLaughlin and Aderem, 1995). This increase in concentration is, however, paralleled by a reduction in the diffusion coefficient, and one has to consider that the orientation of two proteins docked to the plasma membrane may not be favorable for successful interactions. In the case of reversible membrane targeting by myristoyl switches, lipid binding modules, and reversible palmitoylation, discussed in this review, the diffusion in two dimensions after translocation is sufficiently high ($\sim$0.5 μm/s, only $\sim$20-fold slower than in the cytosol) so that protein-protein interactions are indeed markedly increased. Thus, translocation can indeed be used to activate a signaling step, as long as the orientation of the translocated protein permits interactions with the target or activator.

Finally, possibly the most important reason for the large repertoire of covalent lipid modifications and lipid binding modules has to do with the relative evolutionary ease by which enzyme activities can be targeted with different time constants to different cellular locations. By having modular lipid interaction domains and simple amino acid determinants for covalent lipid modifications (such as CaaX boxes or single cysteine residues), the same enzymatic activities can be targeted with different time constants to the same site by simple mutations of the interaction surface or, alternatively, can be targeted to different cellular locations by changing the lipid targeting mechanism. This versatility in timing and localization of membrane targeting is likely one of the main evolutionary driving forces that led to the expansion of the repertoire of membrane-interacting signaling proteins in the vertebrate genome.

Acknowledgments

We would like to thank Drs. Annette Salmeen and Mike Bradshaw for critical reading of the manuscript. Thanks also to the reviewers for their insightful comments.

References

Ames, J.B., Tanaka, T., Stryer, L., and Ikura, M. (1996). Portrait of a myristoyl switch protein. Curr. Opin. Struct. Biol. *6*, 432–438.

Ames, J.B., Ishima, R., Tanaka, T., Gordon, J.I., Stryer, L., and Ikura, M. (1997). Molecular mechanics of calcium-myristoyl switches. Nature *389*, 198–202.

Arbuzova, A., Wang, J., Murray, D., Jacob, J., Cafiso, D.S., and McLaughlin, S. (1997). Kinetics of interaction of the myristoylated alanine-rich C kinase substrate, membranes, and calmodulin. J. Biol. Chem. *272*, 27167–27177.

Augustine, G.J., Santamaria, F., and Tanaka, K. (2003). Local calcium signaling in neurons. Neuron *40*, this issue, 331–346.

Baraldi, E., Carugo, K.D., Hyvonen, M., Surdo, P.L., Riley, A.M., Potter, B.V., O'Brien, R., Ladbury, J.E., and Saraste, M. (1999). Structure of the PH domain from Bruton's tyrosine kinase in complex with inositol 1,3,4,5-tetrakisphosphate. Struct. Fold. Des. *7*, 449–460.

Bergo, M.O., Leung, G.K., Ambroziak, P., Otto, J.C., Casey, P.J., and Young, S.G. (2000). Targeted inactivation of the isoprenylcysteine carboxyl methyltransferase gene causes mislocalization of K-Ras in mammalian cells. J. Biol. Chem. *275*, 17605–17610.

Bergo, M.O., Ambroziak, P., Gregory, C., George, A., Otto, J.C., Kim, E., Nagase, H., Casey, P.J., Balmain, A., and Young, S.G. (2002). Absence of the CAAX endoprotease Rce1: effects on cell growth and transformation. Mol. Cell. Biol. *22*, 171–181.

Beven, L., Adenier, H., Kichenama, R., Homand, J., Redeker, V., Le Caer, J.P., Ladant, D., and Chopineau, J. (2001). Ca2+-myristoyl switch and membrane binding of chemically acylated neurocalcins. Biochemistry *40*, 8152–8160.

Bijlmakers, M.J., and Marsh, M. (2003). The on-off story of protein palmitoylation. Trends Cell Biol. *13*, 32–42.

Bivona, T.G., Perez De Castro, I., Ahearn, I.M., Grana, T.M., Chiu, V.K., Lockyer, P.J., Cullen, P.J., Pellicer, A., Cox, A.D., and Philips, M.R. (2003). Phospholipase Cgamma activates Ras on the Golgi apparatus by means of RasGRP1. Nature *424*, 694–698.

Bolsover, S.R., Gomez-Fernandez, J.C., and Corbalan-Garcia, S. (2003). Role of the Ca2+/phosphatidylserine binding region of the C2 domain in the translocation of protein kinase Calpha to the plasma membrane. J. Biol. Chem. *278*, 10282–10290.

Braunewell, K.H., and Gundelfinger, E.D. (1999). Intracellular neuronal calcium sensor proteins: a family of EF-hand calcium-binding proteins in search of a function. Cell Tissue Res. *295*, 1–12.

Bredt, D.S., and Nicoll, R.A. (2003). AMPA receptor trafficking at excitatory synapes. Neuron *40*, this issue, 361–379.

Brown, D.A., and London, E. (2000). Structure and function of sphingolipid- and cholesterol-rich membrane rafts. J. Biol. Chem. *275*, 17221–17224.

Chen, Z., Otto, J.C., Bergo, M.O., Young, S.G., and Casey, P.J. (2000). The C-terminal polylysine region and methylation of K-Ras are critical for the interaction between K-Ras and microtubules. J. Biol. Chem. *275*, 41251–41257.

Cho, W. (2001). Membrane targeting by C1 and C2 domains. J. Biol. Chem. *276*, 32407–32410.

Choy, E., Chiu, V.K., Silletti, J., Feoktistov, M., Morimoto, T., Michaelson, D., Ivanov, I.E., and Philips, M.R. (1999). Endomembrane trafficking of ras: the CAAX motif targets proteins to the ER and Golgi. Cell *98*, 69–80.

De Matteis, M., Godi, A., and Corda, D. (2002). Phosphoinositides and the golgi complex. Curr. Opin. Cell Biol. *14*, 434–447.

Dolmetsch, R. (2003). Excitation-transcription coupling: signaling by ion channels to the nucleus. Science's STKE, PE4.

Dotti, C.G., and Simons, K. (1990). Polarized sorting of viral glycoproteins to the axon and dendrites of hippocampal neurons in culture. Cell *62*, 63–72.

Ehlers, M.D. (2002). Molecular morphogens for dendritic spines. Trends Neurosci. *25*, 64–67.

El-Husseini Ael, D., and Bredt, D.S. (2002). Protein palmitoylation: a regulator of neuronal development and function. Nat. Rev. Neurosci. *3*, 791–802.

El-Husseini Ael, D., Craven, S.E., Brock, S.C., and Bredt, D.S. (2001). Polarized targeting of peripheral membrane proteins in neurons. J. Biol. Chem. *276*, 44984–44992.

El-Husseini Ael, D., Schnell, E., Dakoji, S., Sweeney, N., Zhou, Q., Prange, O., Gauthier-Campbell, C., Aguilera-Moreno, A., Nicoll, R.A., and Bredt, D.S. (2002). Synaptic strength regulated by palmitate cycling on PSD-95. Cell *108*, 849–863.

Ferguson, K.M., Kavran, J.M., Sankaran, V.G., Fournier, E., Isakoff, S.J., Skolnik, E.Y., and Lemmon, M.A. (2000). Structural basis for discrimination of 3-phosphoinositides by pleckstrin homology domains. Mol. Cell *6*, 373–384.

Fu, H.W., and Casey, P.J. (1999). Enzymology and biology of CaaX protein prenylation. Recent Prog. Horm. Res. *54*, 315–342.

Funamoto, S., Meili, R., Lee, S., Parry, L., and Firtel, R.A. (2002). Spatial and temporal regulation of 3-phosphoinositides by PI 3-kinase and PTEN mediates chemotaxis. Cell *109*, 611–623.

Goldberg, J. (1998). Structural basis for activation of ARF GTPase: mechanisms of guanine nucleotide exchange and GTP-myristoyl switching. Cell *95*, 237–248.

Hilgemann, D.W., Feng, S., and Nasuhoglu, C. (2001). The complex and intriguing lives of PIP2 with ion channels and transporters. Science's STKE, RE19.

Horton, A.C., and Ehlers, M.D. (2003). Neuronal polarity and trafficking. Neuron *40*, this issue, 277–295.

Hurley, J.H., and Meyer, T. (2001). Subcellular targeting by membrane lipids. Curr. Opin. Cell Biol. *13*, 146–152.

Isakoff, S.J., Cardozo, T., Andreev, J., Li, Z., Ferguson, K.M., Abagyan, R., Lemmon, M.A., Aronheim, A., and Skolnik, E.Y. (1998). Identification and analysis of PH domain-containing targets of phosphatidylinositol 3-kinase using a novel in vivo assay in yeast. EMBO J. *17*, 5374–5387.

Jareb, M., and Banker, G. (1998). The polarized sorting of membrane proteins expressed in cultured hippocampal neurons using viral vectors. Neuron *20*, 855–867.

Johnson, D.R., Bhatnagar, R.S., Knoll, L.J., and Gordon, J.I. (1994). Genetic and biochemical studies of protein N-myristoylation. Annu. Rev. Biochem. *63*, 869–914.

Kleuss, C., and Krause, E. (2003). Galpha(s) is palmitoylated at the N-terminal glycine. EMBO J. *22*, 826–832.

Lee, C.H., Della, N.G., Chew, C.E., and Zack, D.J. (1996). Rin, a neuron-specific and calmodulin-binding small G-protein, and Rit define a novel subfamily of ras proteins. J. Neurosci. *16*, 6784–6794.

Lee, S.J., Xu, H., Kang, L.W., Amzel, L.M., and Montell, C. (2003). Light adaptation through phosphoinositide-regulated translocation of *Drosophila* visual arrestin. Neuron *39*, 121–132.

Lemmon, M.A. (2003). Phosphoinositide recognition domains. Traffic *4*, 201–213.

Linder, M.E., and Deschenes, R.J. (2003). New insights into the mechanisms of protein palmitoylation. Biochemistry *42*, 4311–4320.

Macia, E., Paris, S., and Chabre, M. (2000). Binding of the PH and polybasic C-terminal domains of ARNO to phosphoinositides and to acidic lipids. Biochemistry *39*, 5893–5901.

Magee, A.I., Gutierrez, L., McKay, I.A., Marshall, C.J., and Hall, A. (1987). Dynamic fatty acylation of p21N-ras. EMBO J. *6*, 3353–3357.

Malinow, R., and Malenka, R.C. (2002). AMPA receptor trafficking and synaptic plasticity. Annu. Rev. Neurosci. *25*, 103–126.

Maurer-Stroh, S., Eisenhaber, B., and Eisenhaber, F. (2002). N-terminal N-myristoylation of proteins: prediction of substrate proteins from amino acid sequence. J. Mol. Biol. *317*, 541–557.

McLaughlin, S., and Aderem, A.D. (1995). The myristoyl-electrostatic switch: a modulator of reversible protein-membrane interactions. Trends Biochem. Sci. *20*, 272–276.

Meyer, T., and Shen, K. (2000). In and out of the postsynaptic region: signalling proteins on the move. Trends Cell Biol. *10*, 238–244.

Micheva, K.D., Holz, R.W., and Smith, S.J. (2001). Regulation of presynaptic phosphatidylinositol 4,5-biphosphate by neuronal activity. J. Cell Biol. *154*, 355–368.

Micheva, K.D., Buchanan, J., Holz, R.W., and Smith, S.J. (2003). Retrograde regulation of synaptic vesicle endocytosis and recycling. Nat. Neurosci. *6*, 925–932.

Niv, H., Gutman, O., Kloog, Y., and Henis, Y.I. (2002). Activated K-Ras and H-Ras display different interactions with saturable nonraft sites at the surface of live cells. J. Cell Biol. *157*, 865–872.

Oancea, E., and Meyer, T. (1998). Protein kinase C as a molecular machine for decoding calcium and diacylglycerol signals. Cell *95*, 307–318.

Oancea, E., Teruel, M.N., Quest, A.F., and Meyer, T. (1998). Green fluorescent protein (GFP)-tagged cysteine-rich domains from protein kinase C as fluorescent indicators for diacylglycerol signaling in living cells. J. Cell Biol. *140*, 485–498.

Park, S.H., Zarrinpar, A., and Lim, W.A. (2003). Rewiring MAP kinase pathways using alternative scaffold assembly mechanisms. Science *299*, 1061–1064.

Patterson, S.I., and Skene, J.H. (1999). A shift in protein S-palmitoylation, with persistence of growth-associated substrates, marks a critical period for synaptic plasticity in developing brain. J. Neurobiol. *39*, 423–437.

Pawson, T., and Scott, J.D. (1997). Signaling through scaffold, anchoring, and adaptor proteins. Science *278*, 2075–2080.

Pepinsky, R.B., Zeng, C., Wen, D., Rayhorn, P., Baker, D.P., Williams, K.P., Bixler, S.A., Ambrose, C.M., Garber, E.A., Miatkowski, K., et al. (1998). Identification of a palmitic acid-modified form of human Sonic hedgehog. J. Biol. Chem. *273*, 14037–14045.

Pereira-Leal, J.B., Hume, A.N., and Seabra, M.C. (2001). Prenylation of Rab GTPases: molecular mechanisms and involvement in genetic disease. FEBS Lett. *498*, 197–200.

Perisic, O., Paterson, H.F., Mosedale, G., Lara-Gonzalez, S., and Williams, R.L. (1999). Mapping the phospholipid-binding surface and translocation determinants of the C2 domain from cytosolic phospholipase A2. J. Biol. Chem. *274*, 14979–14987.

Pralle, A., Keller, P., Florin, E.L., Simons, K., and Horber, J.K. (2000). Sphingolipid-cholesterol rafts diffuse as small entities in the plasma membrane of mammalian cells. J. Cell Biol. *148*, 997–1008.

Prior, I.A., Harding, A., Yan, J., Sluimer, J., Parton, R.G., and Hancock, J.F. (2001). GTP-dependent segregation of H-ras from lipid rafts is required for biological activity. Nat. Cell Biol. *3*, 368–375.

Prior, I.A., Parton, R.G., and Hancock, J.F. (2003). Observing cell surface signaling domains using electron microscopy. Science's STKE, PL9.

Qanbar, R., and Bouvier, M. (2003). Role of palmitoylation/depalmitoylation reactions in G-protein-coupled receptor function. Pharmacol. Ther. *97*, 1–33.

Raucher, D., Stauffer, T., Chen, W., Shen, K., Guo, S., York, J.D., Sheetz, M.P., and Meyer, T. (2000). Phosphatidylinositol 4,5-bisphosphate functions as a second messenger that regulates cytoskeleton-plasma membrane adhesion. Cell *100*, 221–228.

Robinson, L.J., Busconi, L., and Michel, T. (1995). Agonist-modulated palmitoylation of endothelial nitric oxide synthase. J. Biol. Chem. *270*, 995–998.

Sampo, B., Kaech, S., Kunz, S., and Banker, G. (2003). Two distinct mechanisms target membrane proteins to the axonal surface. Neuron *37*, 611–624.

Santy, L.C., Frank, S.R., Hatfield, J.C., and Casanova, J.E. (1999). Regulation of ARNO nucleotide exchange by a PH domain electrostatic switch. Curr. Biol. *9*, 1173–1176.

Sato, T.K., Overduin, M., and Emr, S.D. (2001). Location, location, location: membrane targeting directed by PX domains. Science *294*, 1881–1885.

Schlessinger, J., and Lemmon, M.A. (2003). SH2 and PTB domains in tyrosine kinase signaling. Science's STKE, RE12.

Shi, S.H., Jan, L.Y., and Jan, Y.N. (2003). Hippocampal neuronal polarity specified by spatially localized mPar3/mPar6 and PI 3-kinase activity. Cell *112*, 63–75.

Simons, K., and Toomre, D. (2000). Lipid rafts and signal transduction. Nat. Rev. Mol. Cell Biol. *1*, 31–39.

Slepnev, V.I., and De Camilli, P. (2000). Accessory factors in clathrin-dependent synaptic vesicle endocytosis. Nat. Rev. Neurosci. *1*, 161–172.

Spang, A. (2002). ARF1 regulatory factors and COPI vesicle formation. Curr. Opin. Cell Biol. *14*, 423–427.

Stenmark, H., Aasland, R., and Driscoll, P.C. (2002). The phosphatidylinositol 3-phosphate-binding FYVE finger. FEBS Lett. *513*, 77–84.

Taylor, C.W. (2002). Controlling calcium entry. Cell *111*, 767–769.

Teruel, M.N., and Meyer, T. (2000). Translocation and reversible localization of signaling proteins: a dynamic future for signal transduction. Cell *103*, 181–184.

Towler, D.A., Adams, S.P., Eubanks, S.R., Towery, D.S., Jackson-Machelski, E., Glaser, L., and Gordon, J.I. (1988). Myristoyl CoA: protein N-myristoyltransferase activities from rat liver and yeast possess overlapping yet distinct peptide substrate specificities. J. Biol. Chem. *263*, 1784–1790.

Tu, Y., Wang, J., and Ross, E.M. (1997). Inhibition of brain Gz GAP and other RGS proteins by palmitoylation of G protein alpha subunits. Science *278*, 1132–1135.

Varma, R., and Mayor, S. (1998). GPI-anchored proteins are organized in submicron domains at the cell surface. Nature *394*, 798–801.

Venkateswarlu, K., Gunn-Moore, F., Oatey, P.B., Tavare, J.M., and Cullen, P.J. (1998). Nerve growth factor- and epidermal growth factor-stimulated translocation of the ADP-ribosylation factor-exchange factor GRP1 to the plasma membrane of PC12 cells requires activation of phosphatidylinositol 3-kinase and the GRP1 pleckstrin homology domain. Biochem. J. *335*, 139–146.

Wedegaertner, P.B., and Bourne, H.R. (1994). Activation and depalmitoylation of Gs alpha. Cell *77*, 1063–1070.

Wientjes, F.B., and Segal, A.W. (2003). PX domain takes shape. Curr. Opin. Hematol. *10*, 2–7.

Zha, J., Weiler, S., Oh, K.J., Wei, M.C., and Korsmeyer, S.J. (2000). Posttranslational N-myristoylation of BID as a molecular switch for targeting mitochondria and apoptosis. Science *290*, 1761–1765.