

14-3-3 proteins: regulation of endoplasmic reticulum localization and surface expression of membrane proteins

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The density and composition of cell surface proteins are major determinants for cellular functions. Regulation of cell surface molecules occurs at several levels, including the efficiency of surface transport, and is therefore of great interest. As the major phosphoprotein-binding modules, 14-3-3 proteins are known for their crucial roles in a wide range of cellular activities, including the subcellular localization of target proteins. Accumulating evidence suggests a role for 14-3-3 in surface transport of membrane proteins, in which 14-3-3 binding reduces endoplasmic reticulum (ER) localization, thereby promoting surface expression of membrane proteins. Here, we focus on recent evidence of 14-3-3-mediated surface transport and discuss the possible molecular mechanisms.

Introduction

Membrane proteins comprise ~30% of the proteome [1]. For many plasma membrane proteins, their density on the cell surface is often a determinant of their overall function in a cell [2]. In addition to transcriptional regulation, surface expression is dictated by various posttranslational steps, including biogenesis, sorting and trafficking, internalization and degradation. Regulation of any given step(s) could therefore result in a significant change of overall expression. For plasma membrane expression, export from ER is the first crucial step. This involves a quality check of proteins for correct folding and/or subunit assembly. ER localization is often achieved by signal sequences, such as a C-terminal KKXX, and an interaction with coat protein (COP) complex I (COPI) (Box 1). Conversely, distinct forward trafficking signals have been identified that promote efficient ER exit (reviewed in Refs [3,4]). These include a diacidic sequence (e.g. DXE) [5,6] that interacts with COP complex II (COPII) [7,8] (Box 1). After exiting the ER, a protein proceeds to the Golgi apparatus. If these proteins have an exposed ER localization signal, they might be recognized by COPI and be returned by retrograde trafficking. Consequently,

when a protein has both types of signal, it is normally found in the ER [9,10].

Studies have linked posttranslational activity to the alteration of protein surface expression, including phosphorylation-mediated downregulation of cell surface receptors by endocytosis [11–13]. The activation of intracellular kinases, including phosphatidylinositol 3-kinase (PI3K) [2,14], protein kinase A (PKA) or protein kinase C (PKC) [15,16], Akt kinase [17–19] and casein kinase II [20], increases cell surface expression of membrane proteins. However, the precise mechanism of the phosphorylation-activated forward transport is not known.

A common effect of phosphorylation is a change in protein–protein interactions. 14-3-3s were the first protein modules to be identified that bind specifically to phosphorylated substrates. The 14-3-3s are highly acidic and abundant in the brain. In humans, there are seven 14-3-3 isoforms (β , γ , ϵ , ζ , η , τ and σ). These are highly homologous proteins that share ~50% amino acid identity and can form either homo- or heterodimers. Evidence from structural studies and sequence analyses indicates that the primary function of 14-3-3 proteins lies in their preferential binding to phosphorylated substrates through their antiparallel bivalent binding sites. 14-3-3 proteins are phosphorylated *in vivo* and the effects on dimerization and target interactions are being investigated [21,22]. 14-3-3 binding to target proteins encompasses two well characterized internal binding motifs, mode I (RSx[S(P)/T(P)]xP) and mode II (Rx Φ x[S(P)/T(P)]xP; Φ represents an aromatic or aliphatic amino acid and x is any amino acid). In addition to these canonical binding motifs, several earlier reports have identified interactions between 14-3-3 proteins and the C termini of target proteins. This characteristic binding has been proposed as mode III [23] and has high binding affinity that is comparable to that of the canonical binding motifs [24,25]. Recently, studies have suggested that 14-3-3 proteins, through binding to phosphorylated motifs, regulate protein expression on the cell surface. Because many 14-3-3 interactions are involved in signal transduction pathways, the coupling of inducible phosphorylation

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Box 1. COPI and COPII in membrane trafficking

The molecular mechanisms underlying membrane trafficking involve the localized assembly of large protein complexes of two major classes (COPI and COPII) surrounding the transport vesicles. COPII complexes select or enrich cargo proteins and transport anterograde vesicles from the ER, whereas COPI complexes mediate retrograde transport of cargo proteins from Golgi and pre-Golgi compartments back to the ER.

Assembly of the COPII complex requires the ordered recruitment of at least five components to the ER membrane. This process has been best characterized in yeast and is initiated by GDP-GTP exchange on the small GTPase Sar1 at the ER membrane. Sar1-GTP binds to Sec23-Sec24, which in turn recruits Sec13-Sec31, thus generating a prebudding complex. Upon vesicle budding, GTP hydrolysis releases coat subunits from budded vesicles. Recent studies have found interactions of specific signals on transmembrane cargos with COPII complexes, including so-called diacidic sequence motifs and dihydrophobic sequence motifs (reviewed in Ref. [71]).

The coat of COPI vesicles is composed of the small GTPase, ADP-ribosylation factor (ARF) and at least seven subunits (α -, β -, β' -, γ -, δ -, ε - and ζ -COP) of a stable cytosolic protein complex, coatomer. Following the activation of ARF, coatomer is recruited to the membrane and its subsequent polymerization drives bud formation. The direct interaction of COPI and dilysine motifs, typically a C-terminal KKXX or KKKXX sequence of type I transmembrane proteins, is required for cargo selection in retrograde transport. Other ER localization signals, such as dibasic motifs, also interact with the COPI complex (reviewed in Ref. [72]).

to surface expression is of great interest. Therefore, here we focus on discussing the evidence and outstanding questions concerning 14-3-3 regulation of membrane protein expression on the cell surface.

Dimeric 14-3-3 interactions and ER export

14-3-3 proteins regulate intracellular protein partitioning [26] and suppress genetic mutants defective in vesicular transport in yeast [27]. Binding to phosphorylated motifs and homo- or heterodimerization are key biochemical features of 14-3-3s. A suggestion of 14-3-3 involvement in forward transport first came from studies on the KCNK3 potassium channel, which showed correlation of 14-3-3 binding with KCNK3 surface expression [28,29]. KCNK3 channels have four putative transmembrane segments flanked by cytoplasmic N- and C-termini. A dibasic ER retention signal near the N-terminus (KR at aa 2-3) interacts with COPI complex. However, the 14-3-3 binding site in KCNK3 is located at the C-terminus. 14-3-3 binding to the C-terminus correlates with both the surface expression of KCNK3 and the loss of its interaction with β -COP, a component of COPI. This has led to the conclusion that 14-3-3 binding releases KCNK3 from the ER [28].

How might the binding of 14-3-3 release a protein from ER localization? One possibility is that 14-3-3s might inhibit the interaction between COPI and ER localization signals. The mechanism of this possible inhibition might include competition in binding overlapping regions, changes in the oligomerization or the conformation of membrane proteins and/or regulation of COPI efficacy. The RKR ER localization signal was found in Kir6.2 potassium channels [30] and this motif, when tetramerized, interacts with 14-3-3 proteins [31]. This interaction could be inhibited by a 14-3-3 binding peptide (R18), a

non-phosphopeptide initially isolated from random peptide library [32], indicative of RKR binding to the same pocket on 14-3-3 as normal substrates.

Identification of a C-terminal SWTY sequence

One mechanism by which 14-3-3 proteins could promote the surface expression of membrane proteins is to enhance forward transport. To search for forward transport signals, a genetic approach was used to screen large random peptide libraries for sequence motifs that could override an ER localization signal [33]. The strategy is outlined in Figure 1. A surface receptor is engineered that localizes in the ER through a known ER localization signal and, if redistributed to the cell surface, it should confer a gain-of-function phenotype (e.g. cell survival). Using a yeast strain that is null for the *trk1* and *trk2* genes, which encode two major yeast potassium transporters, and measuring growth complementation by the mammalian potassium channel Kir2.1, C-terminal sequences that override an upstream RKR ER localization signal were identified. Among these, one group, which is represented by the sequence RGRSWTY-COOH, termed the 'SWTY' motif, interacts with 14-3-3 proteins and confers the highest levels of surface expression of the otherwise ER-localized reporter protein. The SWTY interaction

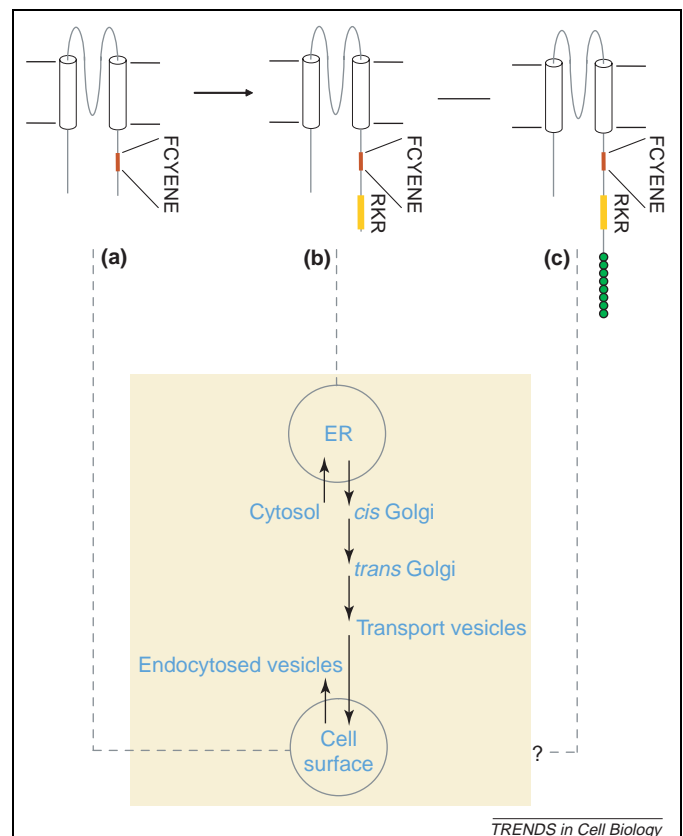


Figure 1. Design of a genetic screen for surface expression signals. (a) The mammalian Kir2.1 potassium channel is expressed on the cell surface; it contains two putative transmembrane segments and an endogenous forward transport signal (FCYENE, red). It therefore complements the yeast *trk1trk2* mutant for growth in low potassium medium. (b) A construct containing the RKR ER localization signal (yellow) and Kir2.1 loses surface expression (no growth complementation). (c) Growth selection of a large collection of random peptides (green) fused to the C-terminus enables identification of those conferring surface expression and growth.

with 14-3-3 proteins is phosphorylation-dependent. A comparable level of surface expression was achieved by replacing the SWTY sequence with the R18 peptide sequence. Because the SWTY motif has no sequence homology to R18, yet binds to 14-3-3, this provides evidence that the 14-3-3 binding overrides RKR activity. SWTY-mediated 14-3-3 binding can override the RKR localization signal placed in monomeric CD4 antigens, dimeric CD8 antigens and tetrameric Kir2.1 potassium channels [33].

The consensus sequence of the SWTY motifs was derived from the genetically isolated sequences that enabled sequence comparison and identification of native SWTY motifs in diverse proteins of different species. The functionality of native SWTY motifs in regulating the surface expression was demonstrated experimentally in both ion channels and G protein-coupled receptors (GPCRs). Because 14-3-3 binding is sufficient to override the ER localization, future investigation should address whether modes I and II internal 14-3-3 binding sequences exert similar activity to that of C-terminal mode III 14-3-3 binding motifs [23,24].

Possible mechanisms of 14-3-3-mediated surface expression

14-3-3 proteins regulate cellular processes by binding to target proteins. No enzymatic activities have been reported, therefore 14-3-3 proteins are likely to mediate their effects on forward transport through specific protein-protein interactions. Previous studies suggest several possible mechanisms by which 14-3-3 interactions exert regulation [34–36]. These mechanisms should be applicable to membrane protein transport. Dimeric 14-3-3 could confer its activity by binding to two different proteins. This scaffolding ability might therefore recruit a range of other activities. Another mode of action could be more direct, by simply competing for interaction with a region that is also recognized by another protein. In this case, the binding of 14-3-3 causes a masking effect, reducing the accessibility to the other binding partner. Third, 14-3-3 might bivalently recognize its target(s) by binding to two sites on one target molecule or by simultaneously binding to two target molecules. As a result, it clamps the target(s) and stabilizes a specific conformation. The evidence supporting these three mechanisms of action is discussed below in the context of membrane protein transport to cell surface.

Scaffolding

14-3-3 proteins might interact with two different proteins (Figure 2a). The structure of 14-3-3 with bound phosphopeptides indicates that each 14-3-3 subunit can independently bind a phosphopeptide [37]. Therefore, it is conceivable that 14-3-3 exerts its function through simultaneously binding to and bridging two different molecules (Figure 2a). Although the scaffolding function of 14-3-3 has not been well characterized for membrane bound proteins, several cytosolic complexes are thought to be bridged by 14-3-3, including those containing the Raf-1 kinase [38–40]. Recent studies have shown that 14-3-3 proteins interact with more than 200 proteins, directly or

indirectly, suggesting 14-3-3 proteins could nucleate heterogeneous protein complexes [41,42].

In a Kir2.1 potassium channel reporter, placing the SWTY sequence downstream of an RKR signal resulted in overriding ER localization and elevated surface expression compared with that of the wild-type Kir2.1 [33]. When RKR is mutated to RAA, the SWTY-induced increase of the surface expression persisted. Thus, the SWTY-elevated surface expression was independent from the ER localization signal. Although it is still possible that 14-3-3 binding to the SWTY motif inactivates the RKR signal by direct interaction (see the following section), the elevated surface expression suggests a gain of function by recruiting protein machineries that could facilitate transport to the cell surface.

Because regulation of surface transport often involves kinase activity, 14-3-3-induced formation of protein complexes between kinases and membrane proteins might facilitate surface transport. In addition to the possibility of recruiting a protein kinase, the affinity pull-down experiments with 14-3-3 proteins suggest that 14-3-3 interacts with proteins that are involved in diverse aspects of membrane trafficking. These include sec23A, sec23B, and sec24C [41], which are members of the core components of the COPII complex that are responsible for ER to Golgi trafficking in a secretory pathway [3]. Furthermore, 14-3-3s directly bind to the plus-end motor protein kinesin, through kinesin light chain-2 (KLC2)

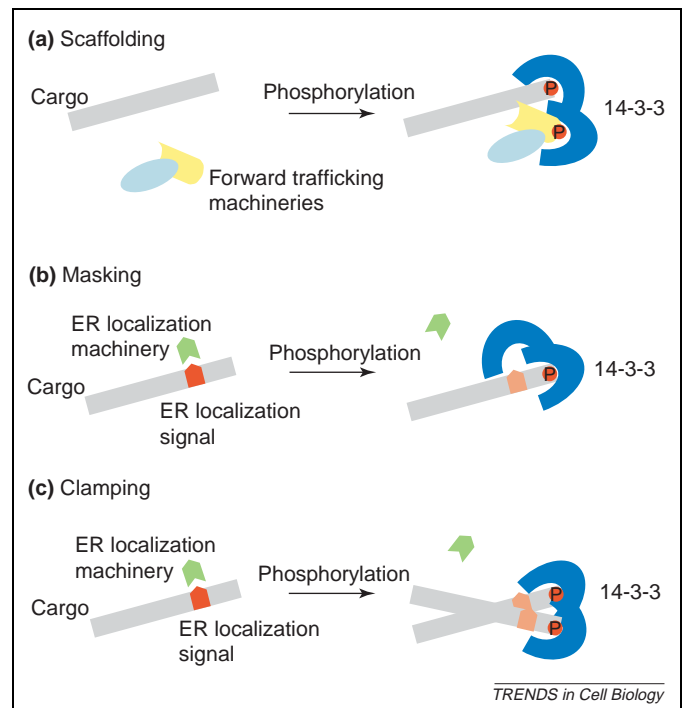


Figure 2. Models of 14-3-3-directed surface trafficking of membrane proteins. The figure depicts the changes conferred by cargo–14-3-3 interaction upon phosphorylation of a C-terminal 14-3-3 binding site. **(a)** Scaffolding. 14-3-3 facilitates the interaction of cargo proteins with forward transport machineries. **(b)** Masking. ER localization signals are masked either by 14-3-3 binding to the phosphorylated target site or by direct binding to an ER localization signal of weaker affinity. **(c)** Clamping. Binding of a 14-3-3 dimer induces a conformation that is unfavorable for ER localization signals, which can be achieved by clustering of targets or relocation from active zones (see main text). This could result in reduced accessibility by ER localization machinery. The color change of ER localization signals in (b,c) represents the functional inactivation of ER localization.

[43,44]. Interestingly, KLC1 and KLC2 interact with the minus-end motor protein, dynein [45], suggesting the coordination of bidirectional motility of cargo along the cellular microtubules. Recent evidence further suggests a direct interaction of COPII with the dynactin (dynein activator) complex, coupling ER export to microtubules [46]. Because COPII can recruit other trafficking machineries, such as soluble *N*-ethyl maleimide-sensitive factor attachment protein receptors (SNARES) [47], these results raise interesting questions for future investigation concerning potential roles of 14-3-3s in recruiting the ER export machineries to membrane proteins. This type of multivalent interaction by an adaptor protein has been seen in glutamate receptor interacting protein 1 (GRIP1), a multi-PDZ domain scaffolding protein that associates with both EphB2 (receptor tyrosine kinase) and KIF5 (kinesin 1) and promotes kinesin-dependent transport of EphB receptors to dendrites in neurons [48,49].

Masking

The 14-3-3-mediated protein translocation to or from the nucleus involves physical masking that is often characterized by linear proximity and, in some cases, overlap of a 14-3-3 binding site and a localization signal. For example, 14-3-3 binding promotes the cytoplasmic translocation of CDC25 members from nuclei, probably by blocking the nuclear localization signal (NLS) that is close to the 14-3-3 binding site [50]. 14-3-3 binding to histone deacetylase (HDAC) also masks a nearby NLS [51]. By contrast, 14-3-3 binding to telomerase reverse transcriptase (TERT) promotes nuclear localization, possibly by obscuring the nuclear export signal (NES) that is located near the 14-3-3 binding site [52]. There is evidence that suggests that 14-3-3 might directly interact with the NLS [53]. The distance from the 14-3-3 binding site to a given trafficking signal might be crucial for 14-3-3 proteins to obscure the interaction of the signal with the trafficking machinery (Figure 2b). Nuclear factor of activated T cells (NFAT) has two NLS sites, one of which is positioned next to a 14-3-3 binding site. The phosphorylation of NFAT by PKA correlates directly with 14-3-3 binding to NFAT and the ability of the NLS to function and, consequently, with the level of transcription [54]. One effective way to test the masking mechanism is to alter the length of the spacer between an NLS (or NES) and a 14-3-3 binding site. In the case of SWTY-mediated surface expression, the activity appears to be independent of the spacer between the RKR ER localization signal and the 14-3-3 binding SWTY site [33]. However, without a more direct structural view of how 14-3-3 orients its binding and the rigidity and conformation of the spacer itself, it is difficult to conclude definitively that there is no masking effect.

Could distant masking occur? This could be the case for KCNK3, in which 14-3-3 binding at the extreme C-terminus of the protein induces a reduction of COPI binding to the dibasic motif that is linearly distant at the N-terminus [28]. Because 14-3-3 is reported to interact with RKR motif [31], it is of interest to determine whether 14-3-3 also interacts with the dibasic (KR) signal in KCNK3.

Direct masking of RKR signal might occur, because 14-3-3 could bind to the RKR motif when oligomerized [31]. The binding of 14-3-3 to RKR is phosphorylation independent. It is possible that this interaction is weak or regulated by other unknown mechanisms because different reporter proteins, including the Kir2.1 tetrameric channel, when fused with the C-terminal sequence of the Kir6.2 containing RKR signal, do not show binding to 14-3-3, as tested by co-immunoprecipitation assay [33]. Consistent with the relatively low affinity, a crosslinker was necessary for 14-3-3 to co-immunoprecipitate with RKR-carrying reporter proteins [31]. In addition, for the γ -aminobutyric acid (GABA)_B receptor, both 14-3-3 and COPI interact with a RSR motif, which is involved in ER localization [55]. Future investigations of other factors affecting 14-3-3 binding to ER localization motifs are needed to clarify the contribution of the absolute affinity, geometry of the binding sites and additional regulatory protein factors in direct masking.

Several other proteins that interact with 14-3-3 through non-canonical binding sequences, such as insulin-like growth factor-1 (IGF-1) receptor, insulin receptor substrate-1 (IRS-1) and vimentin [56,57], might require more than one 14-3-3 binding site. In CDC25B, 14-3-3 first binds to a high affinity site and then to a low affinity site. This sequential engagement appears to be crucial for achieving full activity [58]. These observations raise a possible mechanism underlying SWTY-mediated override of RKR ER retention activity. The SWTY motif binds tightly to 14-3-3 with a dissociation constant (K_D) of 0.17 μ M, compared to the affinities of two 14-3-3 binding sites in Raf-1 (12.8 μ M for Raf259 and >100 μ M for Raf621) [24]. This would enable it to first bind one subunit of the 14-3-3 dimer with high affinity. The recruitment of 14-3-3 through this high affinity site might create the proximity (and hence a high local concentration) of a vacant interaction site on 14-3-3 and enable binding to a low affinity site, RKR. This second binding could therefore prevent COPI from localizing the protein to the ER.

Clamping

There is compelling evidence that 14-3-3s are important allosteric mediators of protein conformational changes (Figure 2c). This is observed by monitoring changes in enzymatic activity as a result of 14-3-3 binding. For example, allosteric clamping of the target protein by 14-3-3 has been demonstrated in AANAT (arylalkylamine-serotonin *N*-acetyltransferase) by a crystal structure in which the 14-3-3 dimer holds its phosphorylated target enzyme in an active conformation [59]. Similarly, 14-3-3s directly bind to and regulate the activity of other enzymes such as tyrosine hydroxylase [60], nitrate reductase [61] and plasma membrane H⁺-ATPase [62]. In proteins that have two phosphorylated 14-3-3-binding sites, it is proposed that one site (called the gatekeeper) first occupies a 14-3-3 binding pocket, followed by occupation of the second site, which cannot bind to 14-3-3 in the absence of the first site binding [63]. The two-site binding mechanism plays a crucial role in stabilizing target proteins in specific conformations. In SWTY signal-containing proteins, if the 14-3-3 binding causes a

conformational change that structurally 'inactivates' the RKR ER retention signal, it could result in release from ER localization. In this regard, it would be interesting to test the clamping effect by a different bivalent binding protein.

Clamping of a membrane protein could result in a conformational change that relocates the RKR ER localization signal. ER localization signals function in specific activity zones in relation to the intracellular membrane leaflet [10]. Therefore, inactivating RKR could be achieved structurally either through an allosteric mechanism or through placement of RKR motifs in a non-functional 'zone'. The zoning mechanism has been seen in several proteins including GABA_B receptor and p35 of the MHC class II complex [64,65].

14-3-3 functions at the cell surface

Steady-state surface expression of membrane proteins is a net balance of surface transport and internalization, recycling and degradation. Does 14-3-3 interaction exert specific effects on target proteins already on the cell surface? 14-3-3 has been found in cytoplasm, nucleus, chloroplasts, various membranes and cytoskeletal and centrosome structures [36]. However, except for cytoplasmic-nuclear partitioning, the significance of differential localization of 14-3-3 proteins remains largely unknown. Because endocytosis is regulated by phosphorylation, 14-3-3 activity at the membrane could have important functions. The 14-3-3-mediated binding to protein C-termini is reminiscent of that mediated by PDZ domains and tetratricopeptide repeat motifs (TPRs) domains [66], both of which are involved in membrane protein localization. For example, phosphorylation of the -2 position serine residue of the β 2AR (adrenergic receptor) type I PDZ ligand sequence by GPCR kinase-5 (GRK5) disrupts interaction with Na⁺-H⁺ exchanger regulatory protein (NHERF)-ezrin-radixin-moesin binding protein (EBP50) and receptor recycling [67]. Furthermore, upon stimulation, certain kinases are translocated to the cell surface and/or can then be activated [68]. Therefore it is interesting to investigate whether there are additional and distinct roles for 14-3-3 at the cell membrane. 14-3-3 prolongs the half-life of target proteins such as Wee-1 [69] and p53 [70]. In the case of the Kir2.1 potassium channel, pulse-chase studies show the increased half-life of Kir2.1 fusion protein on the cell surface if the SWTY motif is fused to the C-terminus. This might also contribute to the increased surface expression of Kir2.1-RKR-SWTY and Kir2.1-RAA-SWTY compared to that of Kir2.1 wild type [33].

Conclusions

The large repertoire of 14-3-3-binding sites and multiple trafficking signals within a single protein suggest the involvement of multiple mechanisms to regulate receptor surface expression, including inhibiting ER localization machinery and engaging forward transport machinery. The phosphorylation-activated 14-3-3-binding sites provide a means for physiological induction of surface expression. Further investigation of these signals in native proteins and by more quantitative methods will

provide more insights into the role of 14-3-3 binding in regulating surface expression of membrane proteins.

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References

- Wallin, E. and von Heijne, G. (1998) Genome-wide analysis of integral membrane proteins from eubacterial, archaean, and eukaryotic organisms. *Protein Sci.* 7, 1029–1038
- Viard, P. *et al.* (2004) PI3K promotes voltage-dependent calcium channel trafficking to the plasma membrane. *Nat. Neurosci.* 7, 939–946
- Barlowe, C. (2003) Signals for COPII-dependent export from the ER: what's the ticket out? *Trends Cell Biol.* 13, 295–300
- Bonifacino, J.S. and Glick, B.S. (2004) The mechanisms of vesicle budding and fusion. *Cell* 116, 153–166
- Nishimura, N. and Balch, W.E. (1997) A di-acidic signal required for selective export from the endoplasmic reticulum. *Science* 277, 556–558
- Sevier, C.S. *et al.* (2000) Efficient export of the vesicular stomatitis virus G protein from the endoplasmic reticulum requires a signal in the cytoplasmic tail that includes both tyrosine-based and di-acidic motifs. *Mol. Biol. Cell* 11, 13–22
- Malkus, P. *et al.* (2002) Concentrative sorting of secretory cargo proteins into COPII-coated vesicles. *J. Cell Biol.* 159, 915–921
- Miller, E.A. *et al.* (2003) Multiple cargo binding sites on the COPII subunit Sec24p ensure capture of diverse membrane proteins into transport vesicles. *Cell* 114, 497–509
- Ma, D. *et al.* (2001) Role of ER export signals in controlling surface potassium channel numbers. *Science* 291, 316–319
- Shikano, S. and Li, M. (2003) Membrane receptor trafficking: evidence of proximal and distal zones conferred by two independent endoplasmic reticulum localization signals. *Proc. Natl. Acad. Sci. U. S. A.* 100, 5783–5788
- Lua, B.L. and Low, B.C. (2005) Activation of EGF receptor endocytosis and ERK1/2 signaling by BPGAP1 requires direct interaction with EEN/endophilin II and a functional RhoGAP domain. *J. Cell Sci.* 118, 2707–2721
- Pearse, B.M. *et al.* (2000) Clathrin coat construction in endocytosis. *Curr. Opin. Struct. Biol.* 10, 220–228
- Cousin, M.A. *et al.* (2001) Protein phosphorylation is required for endocytosis in nerve terminals: potential role for the dephosphins dynamin I and synaptotagmin, but not AP180 or amphiphysin. *J. Neurochem.* 76, 105–116
- Bezzarides, V.J. *et al.* (2004) Rapid vesicular translocation and insertion of TRP channels. *Nat. Cell Biol.* 6, 709–720
- Mangiavacchi, S. and Wolf, M.E. (2004) D1 dopamine receptor stimulation increases the rate of AMPA receptor insertion onto the surface of cultured nucleus accumbens neurons through a pathway dependent on protein kinase A. *J. Neurochem.* 88, 1261–1271
- Scott, D.B. *et al.* (2003) Coordinated PKA and PKC phosphorylation suppresses RXR-mediated ER retention and regulates the surface delivery of NMDA receptors. *Neuropharmacology* 45, 755–767
- Chae, K.S. *et al.* (2005) Akt activation is necessary for growth factor-induced trafficking of functional K(Ca) channels in developing parasympathetic neurons. *J. Neurophysiol.* 93, 1174–1182
- Eyster, C.A. *et al.* (2005) Expression of constitutively active Akt/protein kinase B signals GLUT4 translocation in the absence of an intact actin cytoskeleton. *J. Biol. Chem.* 280, 17978–17985
- Krizman-Genda, E. *et al.* (2005) Evidence that Akt mediates platelet-derived growth factor-dependent increases in activity and surface expression of the neuronal glutamate transporter, EAAC1. *Neuropharmacology* 49, 872–882
- Chung, H.J. *et al.* (2004) Regulation of the NMDA receptor complex and trafficking by activity-dependent phosphorylation of the NR2B subunit PDZ ligand. *J. Neurosci.* 24, 10248–10259

- 21 Woodcock, J.M. *et al.* (2003) The dimeric versus monomeric status of 14-3-3zeta is controlled by phosphorylation of Ser58 at the dimer interface. *J. Biol. Chem.* 278, 36323–36327
- 22 Powell, D.W. *et al.* (2002) Identification of 14-3-3zeta as a protein kinase B/Akt substrate. *J. Biol. Chem.* 277, 21639–21642
- 23 Ganguly, S. *et al.* (2005) Melatonin synthesis: 14-3-3-dependent activation and inhibition of arylalkylamine N-acetyltransferase mediated by phosphoserine-205. *Proc. Natl. Acad. Sci. U. S. A.* 102, 1222–1227
- 24 Coblitz, B. *et al.* (2005) C-terminal recognition by 14-3-3 proteins for surface expression of membrane receptors. *J. Biol. Chem.* 280, 36263–36272
- 25 Wu, M. *et al.* (2006) SWTY-A general peptide probe for homogeneous solution binding assay of 14-3-3 proteins. *Anal. Biochem.* 349, 186–196
- 26 Dougherty, M.K. and Morrison, D.K. (2004) Unlocking the code of 14-3-3. *J. Cell Sci.* 117, 1875–1884
- 27 Gelperin, D. *et al.* (1995) 14-3-3 proteins: potential roles in vesicular transport and Ras signaling in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U. S. A.* 92, 11539–11543
- 28 O'Kelly, I. *et al.* (2002) Forward transport. 14-3-3 binding overcomes retention in endoplasmic reticulum by dibasic signals. *Cell* 111, 577–588
- 29 Rajan, S. *et al.* (2002) Interaction with 14-3-3 proteins promotes functional expression of the potassium channels TASK-1 and TASK-3. *J. Physiol.* 545, 13–26
- 30 Zerangue, N. *et al.* (1999) A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K(ATP) channels. *Neuron* 22, 537–548
- 31 Yuan, H. *et al.* (2003) 14-3-3 dimers probe the assembly status of multimeric membrane proteins. *Curr. Biol.* 13, 638–646
- 32 Wang, B. *et al.* (1999) Isolation of high-affinity peptide antagonists of 14-3-3 proteins by phage display. *Biochemistry* 38, 12499–12504
- 33 Shikano, S. *et al.* (2005) Genetic isolation of transport signals directing cell surface expression. *Nat. Cell Biol.* 7, 985–992
- 34 Bridges, D. and Moorhead, G.B. (2004) 14-3-3 proteins: a number of functions for a numbered protein. *Sci. STKE* 2004, re10
- 35 Fu, H. *et al.* (2000) 14-3-3 proteins: structure, function, and regulation. *Annu. Rev. Pharmacol. Toxicol.* 40, 617–647
- 36 Tzivion, G. *et al.* (2001) 14-3-3 proteins; bringing new definitions to scaffolding. *Oncogene* 20, 6331–6338
- 37 Rittinger, K. *et al.* (1999) Structural analysis of 14-3-3 phosphopeptide complexes identifies a dual role for the nuclear export signal of 14-3-3 in ligand binding. *Mol. Cell* 4, 153–166
- 38 Braselmann, S. and McCormick, F. (1995) Ber and Raffenform a complex in vivo via 14-3-3 proteins. *EMBO J.* 14, 4839–4848
- 39 Conklin, D.S. *et al.* (1995) 14-3-3 proteins associate with cdc25 phosphatases. *Proc. Natl. Acad. Sci. U. S. A.* 92, 7892–7896
- 40 Van Der Hoeven, P.C. *et al.* (2000) 14-3-3 isotypes facilitate coupling of protein kinase C-zeta to Raf-1: negative regulation by 14-3-3 phosphorylation. *Biochem. J.* 345, 297–306
- 41 Pozuelo Rubio, M. *et al.* (2004) 14-3-3-affinity purification of over 200 human phosphoproteins reveals new links to regulation of cellular metabolism, proliferation and trafficking. *Biochem. J.* 379, 395–408
- 42 Jin, J. *et al.* (2004) Proteomic, functional, and domain-based analysis of *in vivo* 14-3-3 binding proteins involved in cytoskeletal regulation and cellular organization. *Curr. Biol.* 14, 1436–1450
- 43 Dorner, C. *et al.* (1999) The kinesin-like motor protein KIF1C occurs in intact cells as a dimer and associates with proteins of the 14-3-3 family. *J. Biol. Chem.* 274, 33654–33660
- 44 Ichimura, T. *et al.* (2002) Phosphorylation-dependent interaction of kinesin light chain 2 and the 14-3-3 protein. *Biochemistry* 41, 5566–5572
- 45 Ligon, L.A. *et al.* (2004) A direct interaction between cytoplasmic dynein and kinesin I may coordinate motor activity. *J. Biol. Chem.* 279, 19201–19208
- 46 Watson, P. *et al.* (2005) Coupling of ER exit to microtubules through direct interaction of COPII with dynactin. *Nat. Cell Biol.* 7, 48–55
- 47 Mossessova, E. *et al.* (2003) SNARE selectivity of the COPII coat. *Cell* 114, 483–495
- 48 Kneussel, M. (2005) Postsynaptic scaffold proteins at non-synaptic sites. The role of postsynaptic scaffold proteins in motor-protein-receptor complexes. *EMBO Rep.* 6, 22–27
- 49 Hoogenraad, C.C. *et al.* (2005) GRIP1 controls dendrite morphogenesis by regulating EphB receptor trafficking. *Nat. Neurosci.* 8, 906–915
- 50 Margolis, S.S. and Kornbluth, S. (2004) When the checkpoints have gone: insights into Cdc25 functional activation. *Cell Cycle* 3, 425–428
- 51 McKinsey, T.A. *et al.* (2001) Identification of a signal-responsive nuclear export sequence in class II histone deacetylases. *Mol. Cell. Biol.* 21, 6312–6321
- 52 Seimiya, H. *et al.* (2000) Involvement of 14-3-3 proteins in nuclear localization of telomerase. *EMBO J.* 19, 2652–2661
- 53 Obsilova, V. *et al.* (2005) 14-3-3 Protein interacts with nuclear localization sequence of forkhead transcription factor FoxO4. *Biochemistry* 44, 11608–11617
- 54 Chow, C.W. and Davis, R.J. (2000) Integration of calcium and cyclic AMP signaling pathways by 14-3-3. *Mol. Cell. Biol.* 20, 702–712
- 55 Brock, C. *et al.* (2005) Assembly-dependent surface targeting of the heterodimeric GABAB Receptor is controlled by COPI but not 14-3-3. *Mol. Biol. Cell* 16, 5572–5578
- 56 Craparo, A. *et al.* (1997) 14-3-3 (epsilon) interacts with the insulin-like growth factor I receptor and insulin receptor substrate I in a phosphoserine-dependent manner. *J. Biol. Chem.* 272, 11663–11669
- 57 Tzivion, G. *et al.* (2000) Calyculin A-induced vimentin phosphorylation sequesters 14-3-3 and displaces other 14-3-3 partners *in vivo*. *J. Biol. Chem.* 275, 29772–29778
- 58 Giles, N. *et al.* (2003) 14-3-3 acts as an intramolecular bridge to regulate cdc25B localization and activity. *J. Biol. Chem.* 278, 28580–28587
- 59 Obsil, T. *et al.* (2001) Crystal structure of the 14-3-3zeta:serotonin N-acetyltransferase complex. a role for scaffolding in enzyme regulation. *Cell* 105, 257–267
- 60 Toska, K. *et al.* (2002) Regulation of tyrosine hydroxylase by stress-activated protein kinases. *J. Neurochem.* 83, 775–783
- 61 Moorhead, G. *et al.* (1996) Phosphorylated nitrate reductase from spinach leaves is inhibited by 14-3-3 proteins and activated by fusicoccin. *Curr. Biol.* 6, 1104–1113
- 62 Kanczewska, J. *et al.* (2005) Activation of the plant plasma membrane H⁺-ATPase by phosphorylation and binding of 14-3-3 proteins converts a dimer into a hexamer. *Proc. Natl. Acad. Sci. U. S. A.* 102, 11675–11680
- 63 Yaffe, M.B. (2002) How do 14-3-3 proteins work?—Gatekeeper phosphorylation and the molecular anvil hypothesis. *FEBS Lett.* 513, 53–57
- 64 Gassmann, M. *et al.* (2005) The RXR-type endoplasmic reticulum-retention/retrieval signal of GABAB1 requires distant spacing from the membrane to function. *Mol. Pharmacol.* 68, 137–144
- 65 Khalil, H. *et al.* (2005) A three-amino-acid-long HLA-DR β cytoplasmic tail is sufficient to overcome ER retention of invariant-chain p35. *J. Cell Sci.* 118, 4679–4687
- 66 Gatto, G.J., Jr. *et al.* (2000) Peroxisomal targeting signal-1 recognition by the TPR domains of human PEX5. *Nat. Struct. Biol.* 7, 1091–1095
- 67 Cao, T.T. *et al.* (1999) A kinase-regulated PDZ-domain interaction controls endocytic sorting of the β 2-adrenergic receptor. *Nature* 401, 286–290
- 68 Anderson, K.E. *et al.* (1998) Translocation of PDK-1 to the plasma membrane is important in allowing PDK-1 to activate protein kinase B. *Curr. Biol.* 8, 684–691
- 69 Wang, Y. *et al.* (2000) Binding of 14-3-3 β to the carboxyl terminus of Wee1 increases Wee1 stability, kinase activity, and G2-M cell population. *Cell Growth Differ.* 11, 211–219
- 70 Yang, H.Y. *et al.* (2003) 14-3-3 σ positively regulates p53 and suppresses tumor growth. *Mol. Cell. Biol.* 23, 7096–7107
- 71 Fromme, J.C. and Schekman, R. (2005) COPII-coated vesicles: flexible enough for large cargo? *Curr. Opin. Cell Biol.* 17, 345–352
- 72 Rabouille, C. and Klumperman, J. (2005) The maturing role of COPI vesicles in intra-Golgi transport. *Nat. Rev. Mol. Cell Biol.* 6, 812–817