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# The ins and outs of syntenin, a multifunctional intracellular adaptor protein

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## Summary

One of the most challenging issues currently facing cell biologists is how signal specificity and compartmentalization is achieved, allowing extracellular stimulation to result in a unique and pre-defined intracellular outcome. For this to occur, intracellular components must be correctly positioned in both space and time. Adaptor molecules, which contain protein-interaction domains, are often involved in the assembly of multimeric complexes that organize intracellular signal-transduction pathways. One such

protein is syntenin, a PDZ-domain-containing molecule that has a surprising variety and diversity of interaction partners. Here we assimilate and discuss current data that support a role for syntenin in regulating transmembrane-receptor trafficking, tumour-cell metastasis and neuronal-synapse function.

Key words: Syntenin, Adaptor, PDZ domain

## Introduction

Over the past decade, studies performed in a range of organisms, from yeast to humans, have demonstrated that adaptor molecules and scaffold proteins are responsible for the organization and assembly of multimeric protein complexes. By driving the association of specific proteins with a variety of interaction domains, the integrity and specificity of a particular signalling pathway is assured. One such interaction module is the PDZ (postsynaptic density protein, disc large and zonula occludens) domain, which generally mediates the assembly of dynamic multi-protein complexes at the membrane by binding to the C-terminus of target proteins. Syntenin is a PDZ-domain-containing protein that was originally identified as a potential melanoma differentiation-associated gene (*mda-9*), the expression of which was induced by interferon  $\gamma$  (IFN $\gamma$ ) treatment (Lin et al., 1998). More recently, there have been many reports that describe interactions between syntenin and a plethora of proteins, indirectly implicating this adaptor molecule in a variety of cellular processes (summarized in Table 1). The surprising diversity of these interactions suggests that syntenin might have flexible cell-type-specific roles, forming unique scaffolds that are dependent on the available intracellular environment.

In this Commentary, we focus on the functional analysis of the PDZ domains in syntenin, which cooperatively bind to a variety of target proteins, and discuss recent literature that support a role for syntenin in regulating the subcellular trafficking of its binding partners, tumour metastases and the integrity of the neuronal synapse.

## The PDZ domains of syntenin: a model for degenerative ligand recognition and cooperative binding

Syntenin is a 32 kDa protein that comprises a 113 amino acid N-terminal domain (NTD) with no obvious structural motifs, followed by two adjacent tandem PDZ domains (PDZ1 and PDZ2)

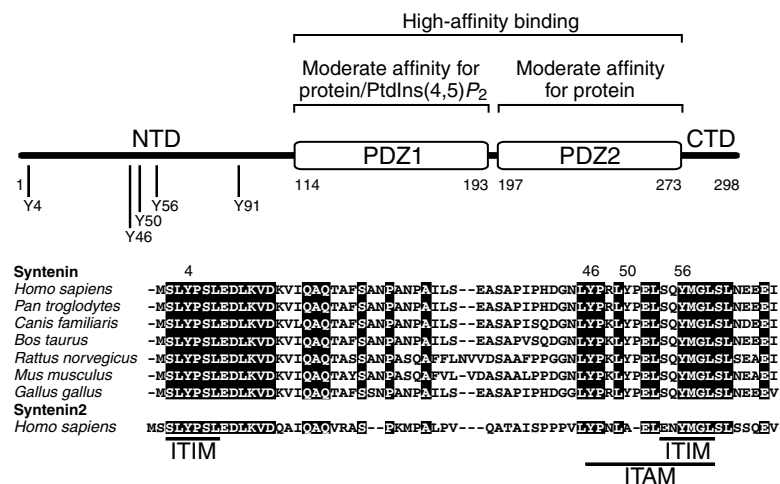
and a short 24 amino acid C-terminal domain (CTD) (Koroll et al., 2001) (Fig. 1). PDZ modules consist of 80-100 residues that form globular, compact domains of 25-30 Å that usually comprise six  $\beta$ -strands ( $\beta$ A- $\beta$ F) and two  $\alpha$ -helices ( $\alpha$ A and  $\alpha$ B). PDZ domains have been grouped according to their peptide-binding specificity, which is determined by side-chains of the peptide at positions  $P_0$  and  $P_{-2}$  (in which  $P_0$  denotes the C-terminal residue of the peptide and  $P_{-n}$  denotes the  $n^{\text{th}}$  residue upstream of  $P_0$ ). Two main peptide-binding motifs of PDZ domains have been recognized: class I, in which the sequence  $P_{-2}P_{-1}P_0$  is [S/T]x $\Phi$  (in which  $\Phi$  represents a hydrophobic residue and x represents any residue; and class II, in which the sequence  $P_{-2}P_{-1}P_0$  is [ $\Phi$ / $\Psi$ ]x $\Phi$  (in which  $\Psi$  represents an aromatic residue). In the canonical model, the carboxylate group of the peptide interacts with conserved residues (GLGF) between  $\beta$ A and  $\beta$ B (the carboxylate binding loop), and  $\beta$ B (primarily residues 5 and 8) and  $\alpha$ B (primarily residue 1) of the PDZ domain.

The PDZ domains of syntenin do not bind a unique sequence, but rather bind to multiple peptide motifs (class I, class II and other sequences) with low-to-medium affinity (Grembecka et al., 2006; Kang et al., 2003b). Degenerative specificity for peptide ligands has also been observed for other PDZ domains, which has significantly complicated PDZ-domain classification (Vaccaro and Dente, 2002). The degenerative binding properties of syntenin have been studied in an extensive series of crystallization and nuclear magnetic resonance (NMR) experiments. The PDZ2 domain of syntenin binds peptides in a canonical orientation; however, binding selectivity of this PDZ domain is determined by the combinatorial use of three binding pockets that bind different structures of the peptide (Cierpicki et al., 2005; Grembecka et al., 2006; Kang et al., 2003b; Kang et al., 2003a). This allows syndecan peptides to bind the PDZ2 domain via  $P_{-1}$  and  $P_{-2}$ , whereas interleukin-5 receptor  $\alpha$  chain (IL5R $\alpha$ ) peptides use  $P_0$  and  $P_{-1}$ . Careful analysis of the PDZ2 domain bound to peptide motifs of syndecan (FYA motif), ephrin B (YKV motif) and neuexin (YYV motif) indicated that

Table 1. Overview of published interaction partners of syntenin

Binding partner	Function	Experimental conditions used for proof of interaction	References
Syndecan	Syndecan recycling	Y2H, SPR, co-IP and colocalization of endogenous, overexpressed and purified proteins, blot overlay, peptide-binding assay	(Grootjans et al., 1997; Grootjans et al., 2000; Zimmermann et al., 2001; Zimmermann et al., 2002; Kang et al., 2003a; Kang et al., 2003b; Grembecka et al., 2006)
Ephrin/EphR	Scaffold; function unknown	Y2H, co-IP of overexpressed or purified proteins, peptide-binding studies, blot overlay	(Torres et al., 1998; Lin et al., 1999; Grootjans et al., 2000; Grembecka et al., 2006)
proTGF $\alpha$	Targeting proTGF $\alpha$ to cell surface	Y2H, co-IP of overexpressed or purified proteins, colocalization after overexpression proTGF $\alpha$	(Fernandez-Larrea et al., 1999)
$\beta$ -neurexin	Function unknown	Y2H, blot overlay, peptide-binding study	(Grootjans et al., 2000; Koroll et al., 2001; Grembecka et al., 2006)
Neurofascin	Function unknown	Y2H, SPR, blot overlay, colocalization after overexpression	(Koroll et al., 2001)
PTPn	Function unknown	Y2H, overexpression co-IP	(Luliano et al., 2001)
IL5R $\alpha$	Activation of SOX4-mediated transcription	Y2H, co-IP of overexpressed or purified proteins, peptide-binding studies	(Geijssen et al., 2001; Kang et al., 2003a; Kang et al., 2003b)
Schwannomin	Subcellular trafficking to plasma membrane	Y2H, co-IP of Sch-1 peptide and purified syntenin, weak endogenous co-IP, blot overlay, peptide-binding assays	(Jannatipour et al., 2001; Kang et al., 2003a)
GluR	Function unknown	Y2H, co-IP of overexpressed or purified proteins, endogenous colocalization	(Hirbec et al., 2002; Hirbec et al., 2003; Enz and Croci, 2003)
SynCAM	Function unknown	Peptide-binding assay	(Biederer et al., 2002)
PICK	Function unknown	Co-IP of purified proteins	(Enz and Croci, 2003)
Unc51.1	Scaffold for linking Unc51.1 to Rab5	Y2H, co-IP and colocalization after overexpression	(Tomoda et al., 2004)
Rab5	Scaffold for linking Unc51.1 to Rab5	Y2H, co-IP and colocalization after overexpression	(Tomoda et al., 2004)
GlyT2	Scaffold mediating interaction with syntaxin 1A	Co-IP after overexpression	(Ohno et al., 2004)
Syntaxin1A	Scaffold mediating interaction with GlyT2	Y2H, co-IP after overexpression	(Ohno et al., 2004)
eIF5A	Regulation of p53 activity	Y2H, co-IP of endogenous, overexpressed or purified proteins, FRET	(Li et al., 2004)
CD6	Scaffold; function unknown	Y2H, co-IP of endogenous or overexpressed proteins, endogenous colocalization	(Gimferrer et al., 2005)
ERC2	Scaffold; function unknown	Y2H, co-IP and colocalization after overexpression	(Ko et al., 2006)
CD63	CD63 internalization	Co-IP of endogenous, overexpressed or purified proteins, peptide-binding assay, colocalization after overexpression	(Latsysheva et al., 2006)
Delta1	Delta1 internalization	Y2H, co-IP after endogenous, overexpression or purified proteins, colocalization after overexpression	(Estrach et al., 2007)
Traf6	Inhibition of IL-1- and TLR4-induced NF- $\kappa$ B activation	Overexpression co-IP	(Chen et al., 2008)
Frizzled 3,7,8	Non-canonical Wnt signalling during <i>Xenopus</i> gastrulation	Blot overlay, SPR, colocalization and co-IP after overexpression	(Luyten et al., 2008)
NG2	Oligodendrocyte precursor migration	Y2H, co-IP after overexpression, endogenous colocalization	(Chatterjee et al., 2008)
Kalirin-7	Function unknown	Yeast two-hybrid interaction	(Penzes et al., 2001)
Neuroglian	Function unknown	Yeast two-hybrid interaction	(Koroll et al., 2001)
SynGAP $\alpha$ 1	Function unknown	Yeast two-hybrid interaction	(Tomoda et al., 2004)
Rab7	Function unknown	Yeast two-hybrid interaction	(Tomoda et al., 2004)
Phospho-inositol lipids	Plasma membrane localization, subcellular trafficking of syndecans	Gel-filtration, binding to PtdIns(4,5)P <sub>2</sub> -containing micelles, SPR, colocalization after overexpression, blot overlay	(Zimmermann et al., 2002; Zimmermann et al., 2005; Mortier et al., 2005; Sugt et al., 2008)

EphR, ephrin receptors; IL5R, interleukin-5 receptor; GluR, glutamate receptors; GlyT2, glycine transporter subtype 2; TGF, transforming growth factor; Y2H, yeast two-hybrid; co-IP, co-immunoprecipitation; SPR, surface plasma resonance (biacore).



**Fig. 1.** The domain structure of syntenin and an overview of conserved N-terminal tyrosine residues. The N-terminal domain (NTD), the PDZ-domain tandem and the C-terminal domain of syntenin. Five tyrosine residues within the NTD (amino acids 1-66) are conserved, as indicated in the lower part of the figure. From these, Y4 and Y56 both consist of consensus ITIM motifs (both SxYxxL), whereas Y46 and Y56 resemble the ITAM motif (YxxLxxxxxxYxxL).

binding induces conformational changes in the  $\alpha$ B helix, which can shift 1.5 Å and is thought to rotate up to 6% (Grembecka et al., 2006). This induced-fit mechanism would optimally position the different binding pockets of the PDZ2 domain for different peptide ligands.

Although most protein ligands show preference for the PDZ2 domain of syntenin, peptides that are derived from the syntenin binding partners IL-5R $\alpha$ , merlin and neuexin were found to interact with the PDZ1 domain with relatively high affinity (syndecan- and ephrinB-derived peptides have a lower affinity for the PDZ1 domain) (Grembecka et al., 2006; Kang et al., 2003b). The PDZ1 domain is also the dominant CD63-interacting domain. The PDZ1 domain of syntenin shows only 26% sequence identity with the PDZ2 domain, but they closely resemble each other structurally. Significant differences do exist, however, which dramatically affect the mode of peptide interaction. Compared with the PDZ2 domain, the PDZ1 domain has a narrow and partially blocked peptide-binding groove that is lined by positively charged clusters. Recent studies indicate that the orientation of the backbone in the PDZ1 domain of a peptide is nearly perpendicular compared with the canonical peptide-PDZ interaction (Grembecka et al., 2006). Peptides bind the first PDZ domain mainly via P<sub>0</sub> and P<sub>-1</sub>, possibly skewing the interactions of this PDZ domain to peptides that have optimal P<sub>0</sub> and P<sub>-1</sub> positions.

More recently, PDZ domains have been found to interact with membrane lipids (reviewed in Zimmermann, 2006). This surprising discovery was first described for syntenin: surface plasmon resonance experiments with liposomes resembling the inner leaflet of the plasma membrane revealed that syntenin directly and strongly interacts with membrane phosphoinositol lipids, and, in particular, with phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P<sub>2</sub>] (Meerschaert et al., 2007; Zimmermann et al., 2002). Subsequent studies also indicate that syntenin 2 binds to PtdIns(4,5)P<sub>2</sub> with high affinity. Syntenin 2 organizes nuclear PtdIns(4,5)P<sub>2</sub> pools, which is crucial for cell survival and proliferation (Mortier et al., 2005). The precise residues that mediate the syntenin-PtdIns(4,5)P<sub>2</sub> interaction are unknown but appear to overlap with peptide-binding

sites; therefore, protein and lipid binding are likely to be mutually exclusive. This was also predicted by a PtdIns(4,5)P<sub>2</sub>-docking model for the PDZ1 domain of syntenin, based on a crystallized PtdIns(4,5)P<sub>2</sub>-PDZ domain of the scaffold protein Tamalin (Sugi et al., 2008). Because PtdIns(4,5)P<sub>2</sub> is not present in some cellular compartments, this would favour syntenin-protein interactions. Conversely, mitogen-induced PtdIns(4,5)P<sub>2</sub> generation would tip the balance towards syntenin-lipid interactions. In this way, the association of syntenin with specific targets could be regulated in a location- and signal-dependent manner (Fig. 1).

Although both PDZ domains can independently interact with various C-terminal peptides, binding studies have indicated that most interactions depend on the complete PDZ1-PDZ2 tandem, suggesting a cooperative binding mode (Grootjans et al., 1997; Fernandez-Larrea et al., 1999; Grootjans et al., 2000; Jannitapour et al., 2001; Geijsen et al., 2001). The individual PDZ domains of syntenin are linked by a short conserved region of five residues, and extensive contact sites between the domains have been observed in crystals and in solution (Cierpicki et al., 2005). These intramolecular interactions are probably important for the relatively fixed orientation of the PDZ1 and PDZ2 domains ( $-5^\circ$ ,  $3^\circ$  and  $-23^\circ$  along the  $x$ -,  $y$ - and  $z$ -axes, respectively), and help to explain why both domains tumble and denature as a single unit (Kang et al., 2003b). The fixed orientation of both PDZ domains and their unique peptide-binding modes, together with the relatively low binding affinities of single PDZ-domain-peptide interactions might all contribute towards the cooperative recognition of (multimerized) target proteins.

#### A role for the N- and C-terminal domains of syntenin?

The NTD and CTD of syntenin encompass the first 113 residues and last 24 residues, respectively. The NTD domain can recruit the transcription factor SOX4 and eukaryotic translation initiation factor 4A (EIF4A) into signalling complexes, and is important for homo- and heterodimerization with syntenin 2 (Geijsen et al., 2001; Koroll et al., 2001; Li et al., 2004). The CTD is required, but not sufficient, for interactions with CD63, and both domains influence the structure and stability of the full-length protein (Cierpicki et al., 2005; Latysheva et al., 2006). Direct evidence that the NTD and CTD regulate interactions between the PDZ domains of syntenin and target proteins is scarce but has been suggested by several studies. For example, tyrosine phosphorylation of the NTD has been reported to prevent the interaction of syntenin with the receptor-type protein tyrosine phosphatase (rPTP) CD148, indicating that post-translational modifications of the NTD affect the interactions between the PDZ tandem and its targets (Harrod and Justement, 2002). Whether NTD phosphorylation affects the structure of the PDZ-domain tandem or oligomerization of syntenin, and thereby rPTP interactions, remains uncertain. The CTD probably contains structural elements that interact with the PDZ tandem, as judged from NMR spectra, but more studies are needed to elucidate exactly how the NTD and CTD contribute to syntenin function.

Endogenous syntenin is tyrosine phosphorylated within its NTD in the haematopoietic-progenitor cell line TF-1 (J.M.B., unpublished observations), and syntenin has been observed to be tyrosine phosphorylated when ectopically expressed in HEK 293T cells (Iuliano et al., 2001). High-stringency analysis reveals four conserved tyrosine residues that are potential phosphorylation sites

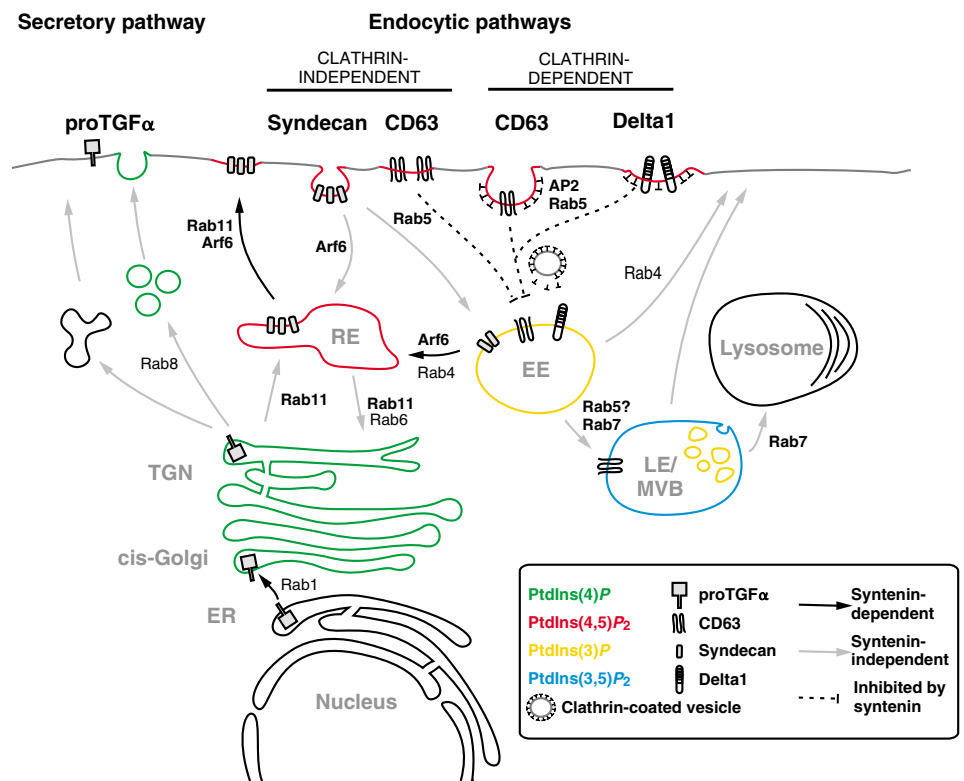
(www.phosphosite.org) (J.M.B., unpublished observations) (Fig. 1). Interestingly, syntenin residues Y46 and Y56 resemble the consensus motif of an immunoreceptor tyrosine-based activation motif (ITAM; consensus  $Yxx[L/I]_x(6-12)Yxx[L/I]$ ), the primary activating signalling domain that is used by classical immunoreceptors. These motifs are phosphorylated by Src family kinases and recruit a variety of SH2-containing proteins, including activating kinases of the Src and Syk/ZAP-70 tyrosine-kinase family, suggesting that this also applies to syntenin (Fig. 1). Interestingly, Y56 also contains an immunoreceptor tyrosine-based inhibition motif (ITIM; consensus  $[I/S/V/L]xYxx[L/I/V]$ ) that is also present at its outmost N-terminus. The 'ITIM-within-an-ITAM' motif is also present in the signal-transducing adaptor molecules  $Ig\alpha$  and DAP12, and in the FERM domain of cytosolic proteins such as schwannomin (Sch-1) and radixin. The exact role of this motif is uncertain, but (partial) phosphorylation events within such motifs might lead to the recruitment of either signal-activating or -inhibiting proteins. Because the NTD has an important role in protein interactions and in the subcellular targeting of syntenin, tyrosine phosphorylation of the NTD might be an important factor in modulating protein interactions and the function of syntenin.

### Syntenin-mediated receptor trafficking

Receptor endocytosis provides a mechanism for the attenuation of signalling by the transfer of receptors to degradative compartments. However, it can also determine signalling output by providing a different combination of downstream effectors at endocytic compartments compared with the plasma membrane. Syndecans are abundant type-I membrane proteins that bear heparin sulphate (HS) side chains on their extracellular domains. These side chains allow them to act as versatile co-receptors that attract and concentrate various growth factors and adhesion molecules at the cell surface,

facilitating interactions with their cognate receptors. The association of syntenin with syndecans was the first reported functional interaction for this adaptor protein (Grootjans et al., 1997). Syntenin mutants that are defective for  $PtdIns(4,5)P_2$  binding trap syndecans in perinuclear recycling endosomes, suggesting an important role for syntenin in syndecan trafficking (Zimmermann et al., 2005). Such endosomal compartments contain Rab11 and Arf6, small GTPases that are implicated in slow endosome recycling. Experiments have revealed that the syntenin-syndecan interaction is not responsible for internalization, but rather for syndecan recycling back to the plasma membrane. Phosphatidylinositol (4)-phosphate 5-kinase [ $PtdIns(4)P$  5-kinase] is the main enzyme responsible for synthesizing  $PtdIns(4,5)P_2$  and is a downstream effector of Arf6.  $PtdIns(4)P$  5-kinase activity and  $PtdIns(4,5)P_2$  turnover, which are controlled by Arf6 activation, are crucial for trafficking through this endosomal recycling pathway back to the plasma membrane. These events led to the hypothesis that syndecan fails to exit from Arf6 recycling endosomes when associated with syntenin in the absence of  $PtdIns(4,5)P_2$ . Indeed, expression of dominant-negative mutants of Arf6 or  $PtdIns(4)P$  5-kinase results in the accumulation of endogenous syntenin and syndecan in perinuclear recycling endosomes. Thus, the release of syndecan from syntenin, which is triggered by increasing  $PtdIns(4,5)P_2$  levels, is required to allow syndecan to traffic back to the cell surface (Fig. 2).

Because syndecan regulates the activity of several transmembrane receptors through its HS chains, it is plausible that syndecan directs HS ligands through a syntenin-regulated recycling pathway. Indeed, the fibroblast growth factor (FGF) receptor accumulates in syndecan-syntenin- $PtdIns(4,5)P_2$  endosomes in an FGF-dependent manner, supporting this hypothesis (Zimmermann et al., 2005). Interestingly, syndecan 4 has recently been demonstrated to be phosphorylated at Ser183, inducing a conformational change in the



**Fig. 2.** Syntenin-dependent subcellular trafficking of receptors and regulation by Rab- and Arf-family GTPases. Black arrows indicate syntenin-controlled trafficking of proTGF $\alpha$  in the secretory route, syndecans and CD63 in the endosomal compartment and tethering of Delta1 at the plasma membrane; grey arrows indicate syntenin-independent trafficking. Inhibitory activity of syntenin for trafficking through a particular route is also indicated. The regulators Rab5, Rab7, Rab11 and Arf6 (indicated in bold) have been reported to bind and/or colocalize with syntenin. The major composition of phosphoinositide lipids that are present in a particular organelle is indicated by the colour scheme in the key. The question mark indicates contradictory results. EE, early endosome; ER, endoplasmic reticulum; LE, late endosome; MVB, multivesicular body; RE, recycling endosome; TGN, trans-Golgi network.



PDZ-domain-binding C2 region (Koo et al., 2006). Surface plasmon resonance data shows that syntenin only binds to the unphosphorylated protein. This suggests that post-translational modification of syndecans might regulate intracellular trafficking via the modulation of syntenin binding. Similarly, the binding of B-class ephrin peptides to syntenin is diminished when they are tyrosine phosphorylated (Lin et al., 1999). Phosphorylation events at the C-termini of proteins could therefore provide a common regulatory mechanism to control the binding of syntenin to specific target proteins.

Evidence that demonstrates a role for syntenin in targeting integral membrane proteins to the cell surface has come from a study of the secretory pathway of pro-transforming growth factor  $\alpha$  (proTGF $\alpha$ ) (Fernandez-Larrea et al., 1999). Membrane-bound proTGF $\alpha$  is a functional ligand in the transduction of mitogenic signals to adjacent cells via epithelial growth factor (EGF) receptor. Determinants in the cytoplasmic tail of proTGF $\alpha$  control its subcellular distribution, and syntenin was found to bind to one of these determinants – the C-terminal amino acid valine (Fernandez-Larrea et al., 1999). As observed for other syntenin-binding partners, both syntenin PDZ domains are required for optimal association, further supporting the tandem PDZ model of high-affinity interaction. Mutation of specific residues in proTGF $\alpha$  that disrupt syntenin binding are also sufficient to result in retention of these mutants in the endoplasmic reticulum. Immunostaining analysis demonstrates that syntenin and proTGF $\alpha$  interact in a perinuclear area that probably coincides with the endoplasmic reticulum, and not at the cell surface (Fig. 2).

In mammalian epidermis, Notch signalling regulates differentiation and has a tumour-suppressor function. Following the binding of one of its ligands (Delta or Jagged), Notch undergoes cleavage of its intracellular domain, which translocates to the nucleus to activate transcription. Via an undefined mechanism, high Delta1 expression promotes keratinocyte cohesiveness. Recently, Delta1 has been described to harbour a conserved PDZ-domain-binding motif in its C-terminus. Mutation of the C-terminal valine (Delta1VA) results in dramatically increased Notch transcriptional activation and epidermal differentiation (Estrach et al., 2007). A two-hybrid screen identified syntenin as a Delta1 binding partner and it was found to localize to cell-cell borders in cultured keratinocytes. Knock-down of syntenin resulted in similar effects as observed with Delta1 mutants that lack C-terminal PDZ-domain binding. Because Delta1 ubiquitylation and internalization are required for Notch signalling, it is plausible that syntenin plays a role in maintaining Delta1 cell-surface expression (Fig. 2). Indeed, RNA interference of syntenin also results in decreased Delta1 plasma-membrane expression (Estrach et al., 2007).

These experiments suggest that syntenin not only has a role in receptor membrane targeting and recycling, but also in the retention or internalization of transmembrane proteins at the cell surface. This is supported by recent work investigating the internalization of tetraspanins, a large family of proteins – each containing four transmembrane domains – that regulate maturation and processing of associated transmembrane proteins as well as their cell-surface activity and internalization (Latysheva et al., 2006). The biological basis for the diverse roles of tetraspanins remains unclear, but it is thought that they regulate the formation of specialized tetraspanin-enriched membrane microdomains (TERMs). CD63, which is ubiquitously expressed, is a tetraspanin that is localized both in late endocytic organelles and on the plasma membrane, and it directly interacts with syntenin (Latysheva et al., 2006). The CD63-syntenin

complex has been observed at the plasma membrane and this interaction is stabilized by the C-terminus of syntenin. It is thought that CD63 undergoes rapid constitutive internalization from the plasma membrane by mechanisms that involve the AP-2 complex and clathrin-dependent endocytosis (Janvier and Bonifacino, 2005). In cells that ectopically overexpress syntenin, CD63 internalization is reduced, and this could be due to competitive inhibition of the CD63–AP-2 association (Fig. 2). Recruitment to CD63 enriches syntenin expression in the TERM, in which it has the potential to interact with a variety of other membrane components, or perhaps cluster or relocate cytoplasmic proteins.

### Syntenin as a modulator of tumour metastasis

Several research groups have recently provided evidence that, in disparate tumours, syntenin might play a role in metastasis. Initially, when differential gene expression between metastatic and non-metastatic cancer-cell lines was compared, Koo and colleagues (Koo et al., 2002) observed that syntenin expression was increased in metastatic cell lines, and this result has been confirmed by several groups (Boukerche et al., 2005; Boukerche et al., 2007; Helmke et al., 2004). Importantly, forced expression of syntenin results in increased migration in non-metastatic cancer cells, and correlates with a more polarized distribution of F-actin and increased pseudopodia formation (Grootjans et al., 1997; Hirbec et al., 2005). The fact that there is no apparent increase in cell adhesion or matrix metalloproteinase (MMP) activity in cells that overexpress syntenin suggests that it is migration itself that is regulated.

But what are the molecular mechanisms that underlie these observations? Increased motility of melanoma cells that express syntenin is observed in wound-healing assays, as is enhanced anchorage-independent growth (Boukerche et al., 2005); these effects are paralleled by extensive dendrite-like processes that contain actin stress fibres. These findings suggest that syntenin interferes with actin dynamics at the plasma membrane. Syntenin colocalizes with focal-adhesion kinase (FAK), a key component of focal-contact structures that can regulate membrane protrusions and cell movement. This colocalization is accompanied by increased FAK activity, which suggests a direct mechanism by which syntenin might increase melanoma metastasis. This also results in increased JNK and p38 MAP kinase activity as well as activation of nuclear factor- $\kappa$ B (NF- $\kappa$ B), all of which seem to play an important role in syntenin-mediated melanoma anchorage-independent growth and motility (Boukerche et al., 2005; Boukerche et al., 2007). In contrast to previous observations in breast and gastric cancer cells (Koo et al., 2002), this is at least partially due to activation of the extracellular-matrix-degrading enzyme MMP2, and probably involves NF- $\kappa$ B-mediated transcriptional regulation of a variety of other genes that modulate cell migration and invasion.

Although taken together these observations are suggestive, they do not formally prove a causal role for syntenin in tumour-cell invasion *in vivo*. However, using a mouse model of tumour growth, forced syntenin expression was found to increase spontaneous melanoma metastasis to the lung (Boukerche et al., 2005). This confirms a causal relationship between syntenin expression and metastatic competence. The precise mechanism by which syntenin recruitment to the plasma membrane results in FAK activation requires further investigation. It does not appear to be caused by a direct interaction but it is possible that syntenin regulates the formation of a macromolecular protein complex, which includes FAK, at the plasma membrane, thereby influencing actin dynamics.

Because more evidence points to a role for syntenin in modulating the metastatic properties of tumours via the modulation of the actin cytoskeleton, future studies should identify which syntenin-driven mechanisms are crucially important for tumour metastases, and how we can interfere with these processes. In the next section, we focus on recent literature that suggests that syntenin can control neuronal function by regulating synaptic integrity and dendritic neurite formation.

### Modulation of neuronal function through control of synaptic integrity

Neuronal synapses are asymmetrical cell-contact sites that have machinery for neurotransmitter release at the pre-synaptic membrane and multiple signalling molecules at the post-synaptic membrane. Underneath these membranes are electron-dense protein matrices that contain cytoskeletal elements and scaffold proteins; these matrices are known as the cytomatrix of the active zone (CAZ) at the pre-synaptic membrane and post-synaptic density (PSD) at the post-synaptic membrane. Within the CAZ, trafficking, docking and fusion to the plasma membrane of vesicles that contain neurotransmitters are regulated, whereas, at the PSD, appropriate receptors are enriched and are partly regulated by recycling-dependent mechanisms. PDZ-domain proteins are major constituents of the CAZ and PSD, and syntenin is present at these sites. These synapses are further supported by a complex array of homo- and heterotypic adhesion molecules that are organized in tight-junction-like adherent sites. The synapse is formed by adhesion molecules that move towards the periphery of the contact site, allowing the sorting of signal-transducing units towards the centre. Interactions between syntenin and adhesion molecules (such as SynCAM, neuexin and neurofascin) and cytoplasmic proteins (such as Sch-1 and PICK1) might all contribute to the establishment and maintenance of proper synaptic structures (Enz and Croci, 2003; Grootjans et al., 2000; Jannatipour et al., 2001; Koroll et al., 2001; Biederer et al., 2002). A recent study indicates that syntenin forms large multimeric complexes at the CAZ, which is initiated by the cell-surface molecule ERC2 (also known as CAST1) (Ko et al., 2006). These intracellular multimeric syntenin complexes might stabilize the asymmetric protein composition at the synaptic membrane and recruit a multitude of intracellular regulators.

Syntenin also interacts with a variety of receptors at the synaptic cleft that regulate neuronal communication, including glutamate receptors and the glycine transporter subtype 2, which removes glycine, an inhibitory transmitter, from the synaptic cleft (Enz and Croci, 2003; Hirbec et al., 2002; Hirbec et al., 2003; Ohno et al., 2004). Glutamate is the principle excitatory neurotransmitter in the CNS and regulates multiple signal-transduction cascades as well as synapse excitability via mechanisms that are located at both pre- and post-synaptic membranes. Fast excitatory responses are regulated via three major classes of ionotropic receptors, and syntenin can associate with an array of these receptors, including the AMPA (an artificial glutamate analogue)-type glutamate receptors GluR1-GluR4, kainate receptors GluR5<sub>2b</sub>, GluR<sub>2c</sub> and GluR6, and metabotropic receptors mGluR4a, mGluR6, mGluR7a and mGluR7b (Enz and Croci, 2003; Hirbec et al., 2002; Hirbec et al., 2003; Enz, 2007). The kainate receptors GluR5 and GluR6 are present at both pre- and post-synaptic membranes, and are highly expressed early in development. They colocalize with syntenin in growth cones and might be important initiators of glutamate-induced membrane protrusions that promote connections in the developing

brain (Hirbec et al., 2005). A role for syntenin in such processes is supported by the observation that ectopic expression of syntenin increases the number of dendritic protrusions in young and mature neurons (Hirbec et al., 2005). Together, these data suggest a role for syntenin in targeting these receptors to correct subcellular locations such as the synapse; further studies, however, are required to critically evaluate whether there is a role for syntenin in maintaining the subcellular locations of these receptors and in synaptic integrity.

### Concluding remarks

The surprising diversity of syntenin-interaction partners suggests that syntenin might have flexible cell-type-specific roles, forming unique scaffolds that are dependent on the intracellular environment or compartment in which it is localized. Thus far, most evidence points to a role for syntenin in subcellular trafficking and signalling of receptors at the plasma membrane and within early endosomal and recycling compartments. Although a multitude of interactions have been established, many of these interactions depend on overexpression systems, and clear functional effects have not been identified. Downregulation of syntenin in cell lines and animals by knockout approaches will be crucial in elucidating syntenin-dependent functions. Most recently, syntenin was found to bind the Wnt-receptor protein Frizzled 7 and to support non-canonical Wnt signalling, which is an important finding, as indicated by the disruption of convergent extension movements during gastrulation after syntenin overexpression and downregulation in *Xenopus laevis* (Luyten et al., 2008).

The specific interplay of syntenin with various binding proteins and/or lipids within a single cell remains largely unknown. Do receptors compete for syntenin binding, and by what mechanisms are interactions regulated? The interaction between the PDZ1 domain and PtdIns(4,5)*P*<sub>2</sub> regulates syntenin-mediated recycling, suggesting that PtdIns(4,5)*P*<sub>2</sub> at high levels can be a dominant PDZ1-domain ligand. The generation of a mutant that can distinguish between lipid and protein interactions would be a valuable tool for evaluating some of these issues. For some ligands, tyrosine phosphorylation of their C-termini has been shown to distinguish their binding of syntenin; however, how these regulatory events shift the repertoire of syntenin-binding proteins under physiological settings remains to be determined.

The roles of the NTD and CTD of syntenin also remain relatively unexplored. Valuable clues into their functions might be derived from structural data of the complete syntenin molecule. Syntenin has been found to interact via its NTD with the SOX4 transcription factor (Geijsen et al., 2001), and it plays a crucial role in homo- and heterodimerization with syntenin 2 (Koroll et al., 2001). Tyrosine phosphorylation of the syntenin NTD might also allow extracellular stimuli to modulate the association of syntenin with its binding partners. The ability of mutant tyrosine variants of syntenin to restore wild-type syntenin functions in cells that have syntenin knocked down would be an ideal setting in which to study this. Moreover, endogenous stimuli that drive tyrosine phosphorylation of syntenin need to be identified before this mechanism can be fully accepted. An important observation is that knockdown of syntenin in tumour cell lines dramatically decreases growth, migration and invasiveness (Meerschaert et al., 2007; Sarkar et al., 2004). This immediately suggests that directly inhibiting syntenin via the use of small-molecule PDZ-domain inhibitors or RNA-interference approaches might provide a novel means of preventing metastatic tumours spreading.

Since the discovery of syntenin as a melanoma-differentiation-associated gene 10 years ago, many new and exciting findings have indicated its involvement in a myriad of cellular functions. The many interaction partners and abundant expression patterns of syntenin will fuel future research in this exciting, fast-moving field, and will be of great importance for our understanding of how adaptor molecules organize intracellular protein complexes in time and space.

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