Hypothesis

A possible role of di-leucine-based motifs in targeting and sorting of the syntaxin family of proteins

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Members of the syntaxin family of proteins are soluble *N*-ethylmaleimide-sensitive factor attachment protein (SNAP) receptors, or SNAREs, which function in vesicle docking and fusion processes [1–3]. The SNARE hypothesis [2,3] has correctly predicted the existence of a family of syntaxin molecules located in various membrane compartments in both the exocytic and the endocytic pathway of yeast [4–9] and mammalian [10–19] cells. Most of the members of the family have now been cloned and localized to different cellular compartments. However, it is not known what primary signals direct the syntaxins to their respective specific cellular locations. It is particularly intriguing how the partition of exocytic versus endocytic locations is achieved.

A recent study from Emr's laboratory [20] demonstrated the existence of a functional di-leucine motif in the yeast syntaxin Vam3p that is essential for AP-3-dependent sorting to the vacuole. Di-leucine motifs are important sorting signals present in endosomal/lysosomal-targeted proteins [21]. Such motifs typically have an acidic residue (D or E) located N-terminal to a pair of leucine residues [22]. In examining the primary sequences of mammalian and yeast syntaxins, we noticed the existence of putative di-leucine-containing motifs in several members of the syntaxin family (Table 1). Interestingly, those syntaxins containing such a putative signal motif have been localized either to the *trans*-Golgi network (TGN) or to the endosome. Amongst the mammalian syntaxins, these include syntaxin 6 (*trans*-Golgi/TGN) [12], syntaxin 7 (endosome) [13], syntaxin 8 (endosome) [29], syntaxin 12 (late endosome) [16] and syntaxin 16 (TGN) [17]. Syntaxin 5 [11], which functions in the early secretory pathway, as well as those at the cell surface (syntaxins 1–4) seem not to contain a recognizable motif in this context. Amongst the yeast syntaxins, putative di-leucine motifs are present in the prevacuolar Pep12p [7] and the vacuolar Vam3p [8], as well as the TGN/endosomal Tlg1p and Tlg2p [9]. Again, the yeast Golgi Sed5p [6] and the surface syntaxins Sso1p and Sso2p [4] do not seem to contain such a signal. The only syntaxin-like molecule whose subcellular localization is incompatible with the presence of a putative di-leucine motif is the yeast endoplasmic reticulum SNARE Ufe1p.

The di-leucine sorting signals have been implicated in endocytosis [21] and bind in vitro to the clathrin coat complex adaptors AP-1 and AP-2 [23]. Recently, a new adaptor complex, termed the AP-3 complex, has been identified. Genetic studies in yeast have revealed a functional role for this new coat complex in cargo-selective transport (such as yeast alkaline phosphatase (ALP)) via an alternative path from the Golgi to the vacuole/lysosome [24,25]. Mutations in AP-3 subunits correspond to classical mutations in *Drosophila* and mice and the phenotypes suggest that protein sorting or biogenesis of storage organelles such as the melanosomes is AP-3-dependent [26]. Indeed, the di-leucine-based motifs in the cytoplasmic tail of the lysosomal integral membrane protein LIMP-II and the melanosome-associated protein tyrosinase have been shown to bind to AP-3 [27]. As mentioned above, Darsow et al. demonstrated that the di-leucine motif of the yeast syntaxin Vam3p is in fact essential for AP-3-dependent sorting [20].

Di-leucine motifs of lysosomal and melanosomal proteins

Table 1
Putative di-leucine motifs in mammalian and yeast syntaxins

Syntaxin	Location	Putative di-leucine motif
Mammalian syntaxins		
Syntaxin 1	presynaptic membrane	–
Syntaxin 2	cell surface	–
Syntaxin 3	cell surface	–
Syntaxin 4	cell surface	–
Syntaxin 5	*cis*-Golgi	–
Syntaxin 6	TGN	^{180}DEQLELV^{186}
Syntaxin 7	endosomal	^{172}EDDLRLI^{178}
Syntaxin 8	endosomal	^{66}DLLKDLL^{72}
		^{87}DRRQNLL^{93}
Syntaxin 10	TGN	–
Syntaxin 11	?	^{53}DENQLLV^{59}
Syntaxin 12	endosomal	^{83}KETNELL^{89}
Syntaxin 16	*trans*-Golgi/TGN	^{202}EDQLVLV^{208}
Yeast syntaxins		
Sed5p	Golgi	–
Sso1p	cell surface	–
Sso2p	cell surface	–
Ufe1p	ER	^{53}KECARLL^{59}
Vamp3p	vacuole	^{154}NEQSPLL^{160}
Pep12p	prevacuolar endosome	^{90}EEIGGLI^{96}
Tlg1p	TGN/endosome	^{174}ENQGQLL^{180}
Tlg2p	TGN/endosome	^{78}DVDDYLL^{84}
		^{201}AENTLLL^{207}

The translated amino acid sequences of all the cDNAs of mammalian and yeast syntaxins were subjected to analysis by the pfscan program of the ISREC Bioinformatics ProfileScan server (http://www.isrec.isb-sib.ch/software/PFSCAN_form.html) and by visual examination. Tabulated are putative di-leucine-containing motifs with an acidic (D or E) amino acid at a position 4 or 5 residues N-terminal to the double leucine (LL, or LV in the case of syntaxins 6, 11 and 16 and LI in the case of syntaxin 7 and Pep12p). A valine or isoleucine can apparently serve the function of the second leucine residue in the leucine pair, as in the case of CD44 and invariant chain a [28]). The numbers indicate the positions of amino acid sequences in the respective parent molecules. The exact membrane localization of syntaxin 11, if any, is not known as immunolocalization with antibody against the endogenous protein has not yet been performed [15,18].

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Table 2
Putative AP-3 binding di-leucine motifs in syntaxins

Potential cargo	Potential AP-3 binding di-leucine motif
Mammalian syntaxin	
Syntaxin 12	K E T N E L L
Yeast syntaxin	
Vamp3p	N E Q S P L L
Tlg2p	A E N T L L L
Vacuolar/lysosomal proteins	
ALP	S E Q T R L V
LIMPII	D E R A P L I
Melanosomal proteins	
Pmel17	G E N S P L L
Tyrosinase	E E R Q P L L
P-protein	K E D T P L L
Consensus	* E X * X L L

Di-leucine-containing motifs of selected mammalian and yeast syntaxins are tabulated with that of vacuolar/lysosomal proteins alkaline phosphatase (ALP), LIMPII, the melanosomal proteins Pmel17, tyrosinase and P-protein and the yeast vacuolar protein Vamp3p as in Odorizzi et al. [26] and Darsow et al. [20]. The consensus requirement of *EX*XLL as derived by Darsow et al. [20] (where * denotes a bias towards charged or polar amino acids and X can be any amino acid) is also satisfied by the putative di-leucine-based motifs of mammalian syntaxin 12 and yeast Tlg2p.

experimentally delineated to be AP-3-dependent (ALP, LIMP-II and tyrosinase) and melanosomal proteins suspected to be so (Pmel17 and P-protein) are tabulated in Table 2 together with the AP-3 binding di-leucine motif of Vam3p [26]. A consensus of *EX*XLL was derived by Darsow et al. [20] (where * denotes a bias towards charged or polar amino acids and X can be any amino acid). We could also add to this table the putative di-leucine based motifs of mammalian syntaxin 12, and yeast Tlg2p. In these cases, the di-leucine motifs match that of the consensus.

Syntaxins (except for syntaxin 11 [15]) are transmembrane proteins with a C-terminal hydrophobic tail anchor [1,11]. Their N-terminal region would thus be exposed to the cytoplasm. It is therefore logical that such a motif is present in endosomal syntaxins to target these molecules to the endocytic pathway upon their exit from the Golgi. This can be achieved either via AP-1-coated vesicles to the late endosomes from the TGN or via AP-2-mediated endocytosis from the cell surface. That such mechanisms are being utilized is implied by the prominent presence of these di-leucine motifs amongst the endosomal syntaxins and the absence of these motifs amongst syntaxins of the early secretory pathway and the cell surface.

It is again interesting to note, in apparent agreement, the presence of a putative AP-3-dependent signal motif in syntaxin 12, amongst the known mammalian syntaxins. Syntaxin 12 is likely concentrated in a late endosomal/prelysosomal compartment [16]. Whether its putative di-leucine motif binds to AP-3 and whether the subcellular targeting and sorting of syntaxin 12 is AP-3-dependent remains to be experimentally determined. Likewise, it remains to be experimentally determined whether any of the putative di-leucine motifs found in endosomal syntaxins are functional. There may well be other signal motifs and targeting mechanisms pertaining to this fascinating family of molecules which await identification. These di-leucine motifs, however, give us a good clue of where to begin.

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