PROTEIN TRANSLOCATION

In or out?

The ability to predict the structure of a protein from its amino-acid sequence is a long-sought-after, but elusive, goal. However, Hessa *et al.* have brought us one step closer to the dream with their recent work on the sequence requirements of protein transmembrane helices.

Membrane proteins are vitally important to the integrity and functions of a cell, and to successfully integrate into the complex environment of the membrane, a protein must have a similarly complex structure, the nature of which is compatible with the membrane environment. Most membrane proteins have a bundle of tightly packed α-helices that span the width of the membrane and that are predominantly hydrophobic in character.

To fold successfully inside the cell such membrane proteins must insert into, and fold within, the endoplasmic reticulum (ER) membrane as they are being translated. This insertion is mediated by the Sec61-translocon protein complex that provides a channel through which the translating protein is conducted. It can translocate polypeptides into the aqueous environment of the ER lumen or can move them laterally into the lipid environment of the ER membrane. But what factors determine whether the translocon inserts a polypeptide into the membrane or pushes it out into the lumen?

In *Nature*, Hessa *et al.* have begun to answer this question by carrying out extensive quantitative studies of translocon-mediated protein insertion. They systematically designed a large set of transmembrane sequences and measured the efficiency of their membrane integration by Sec61. Analysis of these data was used to assess the contributions of individual amino acids to the efficiency of membrane insertion, and a thermodynamic scale was established that measured the energy that was required for the membrane insertion of each amino acid. In fact, the authors found that these biological measurements closely correlated with previously established biophysical measurements for inserting individual amino acids into membrane environments.

By carefully manipulating the amino-acid sequences of their experimental transmembrane segments, Hessa *et al.* showed that the overall hydrophobicity of the segment was not the only determinant of membrane insertion. The position of particular residues within the segment had an important role in its membrane insertion, and specific residues 'preferred' to lie in specific regions of the segment depending on the compatibility of

MEMBRANE TRAFFICKING

Actin' out of control

The Arp2/3 complex has a fundamental role in the regulation of actin assembly at endocytic sites. In yeast, Pan1 is a key regulator of Arp2/3 that is known to be essential for endocytosis and actin organization, but the intricate details of the molecular mechanisms of Arp2/3 regulation at endocytic vesicles have eluded researchers — until now. Toshima *et al.* report in *Nature Cell Biology* that the kinase Prk1 shuts off Arp2/3-mediated actin polymerization during endocytosis by directly phosphorylating and inactivating Pan1.

Using site-directed mutagenesis, the authors showed that a mutant Pan1 protein that is resistant to Prk1-targeted phosphorylation produced large actin clumps and endocytic defects that were similar to those previously seen in Prk1- and Ark1-mutant cells (Ark1 is a Prk1-related kinase). Further analysis revealed that constitutive dephosphorylation of Pan1 caused filamentous (F)-actin structures to associate with endocytic components.

Loss-of-function studies had previously identified Pan1 as a downstream target of Prk1. Toshima *et al.* showed that Prk1 directly phosphorylates Pan1 at its N terminus to suppress its function as an Arp2/3 activator. Phosphorylation of Pan1 probably precludes its association with F-actin, thereby preventing it from activating Arp2/3, as a Pan1 truncation mutant that was defective in F-actin binding also lost its ability to activate Arp2/3 both *in vitro* and *in vivo*. Structure–function analysis revealed that Pan1 contains a Wiskott–Aldrich syndrome protein (WASP) homology-2 (WH2)-like motif that is necessary for Arp2/3 activation. However, in contrast to previously studied WH2 domains, the Pan1 WH2-like motif is unique in that it does not bind globular (G)-actin, but instead binds to F-actin.

So are the actin clumps that were observed in cells expressing phosphorylation-resistant Pan1 a result of uncontrolled actin polymerization? Consistent with unphosphorylated Pan1 being hyperactive, the authors demonstrated that mutating the F-actin-binding sites in constitutively unphosphorylated Pan1 was sufficient to suppress the formation of actin clumps and the associated endocytic defects. This also confirmed the absolute requirement for F-actin binding to Pan1 prior to Arp2/3 activation.

Toshima *et al.* propose an elegant model whereby, at the onset of endocytosis, Pan1 associates with endocytic proteins at the plasma membrane to activate the Arp2/3 complex and

their physical properties with the membrane environment. Manipulating the ability of the transmembrane segment to form a helical structure by inserting proline residues at various positions also showed that α-helix formation is vital for proper membrane insertion.

Taken together, the results indicated that the translocon recognizes transmembrane helices by allowing the direct interaction of the transmembrane segment and the surrounding membrane lipids. In fact, the authors suggest that the translocon has a dynamic structure that allows the translocating peptide to 'sample' the translocon–membrane interface, thereby ensuring that the physical properties of the polypeptide help to direct its fate. The authors are hopeful that, with more work on other factors that could influence membrane insertion, their results might be used in the future to help predict the membrane-insertion efficiency of natural polypeptide segments.

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promote actin polymerization. Phosphorylation of Pan1 by Prk1 results in the release of Pan1 from the endocytic machinery as it can no longer bind to F-actin, and so this inhibits its Arp2/3-activator function. Presumably, Pan1 then joins the same machinery as other endocytic components in preparation for starting a new cycle.

Pan1, although an important component of receptor-mediated endocytosis, is not the only activator of the Arp2/3 complex. The authors have already shown that there is considerable overlap between the Pan1 phenotype and phenotypes that are caused by mutations in the WASP-related protein Las17 — a known Arp2/3 activator. It would certainly be interesting to see whether Las17 is also regulated by Prk1-mediated phosphorylation. However, the extent to which the findings reported by Toshima *et al.* using yeast will translate to more complex organisms remains to be seen, and is certainly something to keep in mind.

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CYTOSKELETON

Small changes, big response

Neuronal Wiskott–Aldrich syndrome protein (N-WASP) activates the Arp2/3 complex, which stimulates actin polymerization. N-WASP activity is repressed by intramolecular interactions whereby the N-terminal polybasic (B) motif and GTPase-binding domain (GBD) inhibit the activity of the C-terminal Arp2/3-stimulating VCA domain. Such autoinhibition is relieved by the binding of phosphatidylinositol 4,5-bisphosphate ($PtdIns(4,5)P_2$) and Cdc42•GTP to the B motif and GBD, respectively. Papayannopoulos *et al.* have discovered that $PtdIns(4,5)P_2$ binds to the B domain in a multivalent and cooperative manner. This means that, above a certain threshold, small increases in the concentration of $PtdIns(4,5)P_2$ confer a switch-like activation on N-WASP.

A polypeptide that corresponded to the GBD and B motif bound most strongly to $PtdIns(4,5)P_2$ from a panel of lipids. Deletion analysis narrowed the region down to a 15-residue fragment (encompassing the B motif) that contained 10 basic residues (mainly lysines), and Papayannopoulos *et al.* showed that this high positive-charge density was required for binding.

The authors then showed that the B motif bound $PtdIns(4,5)P_2$ with higher affinity when it was present at higher density, so they measured the influence of this effect on N-WASP-mediated actin polymerization. Activation, even more than binding, of N-WASP depended on $PtdIns(4,5)P_2$ density, and only occurred *in vitro* above a steep threshold.

The apparent affinity of $PtdIns(4,5)P_2$ for the B motif alone seemed higher than for the full-length form. The authors proposed that the B motif is occluded by autoinhibition in the full-length form of N-WASP, and that, if several adjacent $PtdIns(4,5)P_2$-binding sites are occluded by autoinhibitory interactions, cooperativity of $PtdIns(4,5)P_2$ binding might increase the apparent affinity of subsequent $PtdIns(4,5)P_2$ binding by disrupting autoinhibitory interactions. This proved to be correct. Adding Cdc42 also decreased the N-WASP-activation thresholds, in keeping with Cdc42 relieving the autoinhibitory interactions. Adding both $PtdIns(4,5)P_2$ and prenylated Cdc42 (to mimic its membrane localization) further activated N-WASP.

Next, Papayannopoulos *et al.* investigated the motility rates of partially purified endosomal vesicles to assess actin-mediated vesicle motility. They depleted endogenous N-WASP, substituted N-WASP variants and artificially induced vesicle motility. Wild-type N-WASP could mediate vesicle motility, but deleting the B motif or some of the basic lysine residues within it couldn't reconstitute motility. But when the number of lysines was increased, motile vesicles with longer actin-containing comet tails were generated, and moved faster than the wild-type-N-WASP-driven vesicles. A significant amount of N-WASP was also activated at resting levels of $PtdIns(4,5)P_2$ *in vivo* when extra lysines were present in the B motif. Consistent with this, the construct containing extra lysines showed a lower activation threshold and increased threshold steepness, most likely because the multivalency (the number and the affinity) of $PtdIns(4,5)P_2$-binding sites was increased.

The finding that the polybasic region of N-WASP binds to $PtdIns(4,5)P_2$ in a multivalent manner, and is involved in the sharpness of the $PtdIns(4,5)P_2$-mediated transition, provides another example of cooperative activation in biology. This sharp activation threshold probably confers on N-WASP the ability to respond to subtle, signal-induced changes in the local concentrations of $PtdIns(4,5)P_2$.

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WEB SITE

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