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.

Myrto Raftopoulou, Assistant Editor,
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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)P₂) and Cdc42•GTP to the B motif and GBD, respectively. Papayannopoulos *et al.* have discovered that PtdIns(4,5)P₂ 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)P₂ 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)P₂ 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)P₂ 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)P₂ density, and only occurred *in vitro* above a steep threshold.

The apparent affinity of PtdIns(4,5)P₂ 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)P₂-binding sites are occluded by autoinhibitory interactions, cooperativity of PtdIns(4,5)P₂ binding might increase the apparent affinity of subsequent PtdIns(4,5)P₂ 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)P₂ 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)P₂ *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)P₂-binding sites was increased.

The finding that the polybasic region of N-WASP binds to PtdIns(4,5)P₂ in a multivalent manner, and is involved in the sharpness of the PtdIns(4,5)P₂-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)P₂.

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WEB SITE

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