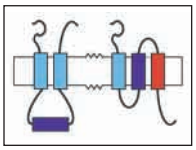


Transmembrane protein biosynthesis

Getting transmembrane (TM) domain(s) into the right orientation during biosynthesis is clearly essential for correct function of most integral membrane proteins. But what are the factors that ensure this? And how successful are current algorithms at predicting membrane protein topology from amino acid sequence alone – particularly if the protein has multiple TM segments? In a Commentary on p. 2003, Carolyn Ott and Vishwanath Lingappa review work that is shedding light on the role of intra- and inter-protein interactions in regulating integral membrane protein topology. Intraprotein interactions include those involving 'strong orientation effectors', such as TM domain 8 of the model protein band 3, which forces the integration of the preceding TM domain. Correct orientation of other proteins (e.g. IgM) requires interprotein interactions, which involve trans-acting factors such as translation accessory factors (TrAFs) or the stop transfer effector (STE) receptor. Indeed, the complexity of membrane protein biogenesis underlines the need for caution when topology prediction algorithms are used, particularly given that several proteins are synthesized in several distinct topological forms.

Different integrin, different signal

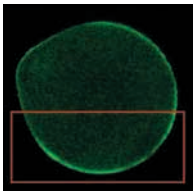
The binding of ligands such as fibronectin to cell surface integrins produces a variety of intracellular signals. An attractive idea is that different integrin subtypes generate different signals, but strong evidence for this has been lacking. Shu Chien and co-workers now demonstrate that two integrin subtypes can indeed activate distinct signalling pathways (see p. 2199). They show that, when CHO cells are plated on fibronectin, integrin-β1-expressing cells produce lamellipodia and activate Rac – a Rho-family GTPase implicated in induction of lamellipodia. By contrast, integrin-β3-expressing cells generate actin stress fibres and activate Rho, which is thought to control stress fibre formation. Intriguingly, the authors found that expression of a chimeric construct in which one of the extracellular domains of integrin β1 was replaced by the corresponding domain from integrin β3 produced stress fibres and an increase in Rho activity. Their findings thus indicate not only that the two integrins signal differently in response to fibronectin but that differences in the extracellular region rather than the intracellular region are responsible.

Genetic selection of endothelial cells from stem cells

Endothelial cell transplantation has immense therapeutic potential. Not only could it improve tissue grafting after injury, it could also be used to treat patients with vascular defects, supplying circulating endothelial progenitor cells (EPCs) for formation of new blood vessels. Unfortunately, isolation and expansion of EPCs ex vivo is a slow process; alternative methods that allow generation of large numbers of EPCs would be a huge asset. Gilles Pagès and co-workers now present just such a method (see p. 2075). They have developed a genetic approach for selecting endothelial cells from differentiating embryonic stem (ES) cells. The authors established ES cells expressing a puromycin-resistance gene under the control of the endothelium-specific *Tie-1* promoter – having shown that it drives endothelium-specific expression of a GFP reporter. Pagès and co-workers show that, after expansion, differentiation and puromycin selection, the resulting cells express characteristic endothelial markers, such as VE-cadherin and ICAM-2. Moreover, they find that these cells can participate in neovascularization in vivo, concluding that their technique has potential for pro-angiogenic therapy.

Non-neuronal role for CDK5 in cell adhesion

CDK5 is a cyclin-dependent kinase expressed primarily in neurons, in which it functions downstream of Rac in control of neurite outgrowth. But the kinase is also expressed in some non-neuronal tissues, including muscle and the lens. What is its function in these cells? Peggy Zelenka and co-workers have investigated non-neuronal roles of CDK5 by analysing the adult mouse lens and stably transfected lens epithelial cells (see p. 2109). They find that CDK5 is expressed in the cytoplasm of differentiating fibre cells, especially along the lateral membranes and at the basal tips, which contain elaborate junctional complexes. Speculating that this reflects a role for CDK5 in cell adhesion, the authors assessed the performance of CDK5-expressing epithelial cells in spreading and adhesion assays. They demonstrate that these cells exhibit increased attachment to fibronectin matrices and elevated spreading rates but that cell-cell adhesion and association of N-cadherin with the cytoskeleton are reduced. Zelenka and co-workers conclude that CDK5 differentially regulates cell-cell and cell-matrix adhesion, suggesting that this is important for coordination of elongation and adhesion during lens fibre cell differentiation.



PtdIns(4,5)P_2 dynamics at fertilization

Fertilization of mammalian eggs triggers a series of sustained intracellular Ca^{2+} oscillations required for resumption of meiosis and exocytosis of cortical granules. The release of Ca^{2+} from intracellular stores is a consequence of phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] turnover. One might therefore expect to see reduced levels of plasma membrane PtdIns(4,5)P_2 after fertilization. Instead, it seems that they go up (see p. 2139). Guillaume Halet and co-workers have used GFP fusion proteins containing a pleckstrin-homology domain that binds to PtdIns(4,5)P_2 to monitor its dynamics in eggs. They find that fertilization produces a net increase in PtdIns(4,5)P_2 levels around the vegetal pole after the initial rise in Ca^{2+} concentration. This increase can be blocked by Ca^{2+} buffers and specific inhibitors of Ca^{2+}-dependent exocytosis. In addition, it can be blocked by 30 μM wortmannin (which inhibits PtdIns(4,5)P_2 synthesis) but not 100 nM wortmannin (which inhibit PI 3-kinase). Carroll and co-workers therefore propose that fertilization increases PtdIns(4,5)P_2 levels through Ca^{2+}-dependent cortical granule exocytosis. This could supply substrate/enzymes for synthesis of PtdIns(4,5)P_2, which might participate in membrane retrieval or actin remodelling after exocytosis.

Sticky Wicket – worthy of a place at the table

A biochemist describes herself as a chemical biologist, a pathologist now claims to work on genomics and everyone else seems to work in some fascinating cross-over discipline. Caveman is worried that describing himself as a 'cell biologist' does not sound exciting enough in this company. Fortunately, the alien inside him has a solution (see p. 1999).

In the next issue of JCS