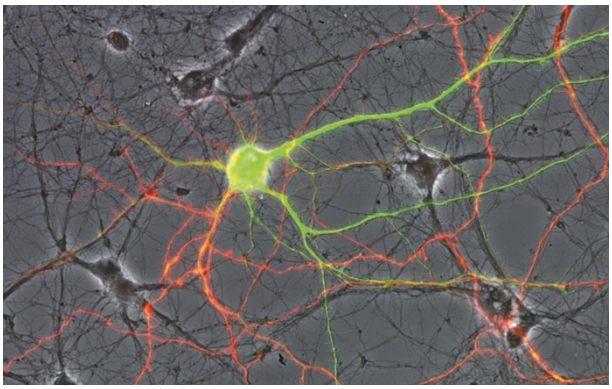
Axonal polarization: selective delivery and retention

Polarisation of the cell surface into structurally and functionally discrete sub-domains is a characteristic feature of most eukaryotic cells. Two general mechanisms are thought to contribute to the generation and maintenance of cell-surface polarization: proteins are either selectively delivered to particular surface regions, or delivered equally to all regions and selectively retained at the appropriate cell-surface domains. The axon and dendrite are functionally distinct polarized domains of a neuron. Current evidence favours a role for selective retention in axonal polarization. Now, a study by Sampo and colleagues (*Neuron* **37**, 611–624 (2003)) suggests that in addition to selective retention, selective delivery also has an important role in axonal polarization.

To gain insights into the processes that govern axonal polarization, the authors examined trafficking of Vamp2 and NgCAM, two proteins that are selectively transported to the axonal cell surface. The authors first reasoned that preferential endocytosis from the dendritic cell surface may result in the axonal distribution of proteins. Although Vamp2-containing endosomes were found in dendrites, NgCAM was not present in these endosomes in the majority of hippocampal neurons. Moreover, an endocytosis-deficient Vamp2 mutant was no longer selectively transported to the axon, but rather, had the distribution of a protein that is transported in a non-polarized fashion. In contrast, disruption of the endocytosis motif in NgCAM and another axonal protein, L1, did not prevent the axonal distribution of either protein (see Figure). These findings strongly suggest that selective retention of Vamp2 at the axonal surface maintains the axonal polarization of Vamp2.

In contrast to Vamp2, the extracellular domain (rather than the cytoplasmic domain) of NgCAM contains essential determinants for polarization. In particular, it contains five fibronectin type III-like (FnIII) repeats in the extracellular domain that are necessary for selective axonal targeting. When grafted onto a related, but unpolarized, protein such as NrCAM, these five FnIII repeats were sufficient to redistribute NrCAM to the axonal surface, suggesting that selective sorting and delivery result in the axonal distribution of NgCAM.



Role of the NgCAM ectodomain in polarized transport. A fluorescence image showing soluble green fluorescent protein (GFP; green, axons and dendrites) and a chimaera of the NgCAM ectodomain and the cytoplasmic domain of the unpolarized protein CD8 (red, axonal surface, but not dendrites). Figure adapted from Sampo *et al.* © (2003), with permission from Elsevier Science.

These studies demonstrate that polarization of proteins to the axon relies on both selective retention and selective delivery. It remains unclear exactly how Vamp2 is preferentially retained at the axonal surface and how selective sorting of NgCAM is accomplished. Future work will undoubtedly provide further insights into these and other aspects of neuronal polarization.

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describe the discovery of a membrane-permeable Ku70-derived peptide that can block certain types of apoptosis[11].

Using a yeast-based functional screen, Sawada *et al.*[10] identify Ku70 as an inhibitor of Bax function. Overexpression of Ku70 in mammalian cell lines efficiently blocked apoptosis induced by a number of stimuli that are known to work through Bax; it did not, however, abrogate apoptotic responses that are not mediated by mitochondria. In contrast, depletion of Ku70 rendered cells more sensitive to apoptotic stimuli known to work through Bax. The specificity of Ku70 for Bax is further emphasized by the finding that Ku70 did not block Bak-mediated cell death in Bax-knockout cells. So how does Ku70 inhibit Bax function? A previously identified protein, Bax inhibitor-1, inhibited Bax-mediated apoptosis, but did not associate with Bax itself and probably functions at a downstream step[12]. In contrast, binding of Ku70 to Bax is dependent on the N-terminal 53 amino acids of Bax. Overexpression of Ku70 efficiently prevented translocation of Bax from the cytosol to mitochondria under conditions of apoptotic stress. Remarkably, a large proportion of Bax was found in complex with Ku70 in healthy cells. One possibility is that Ku70 may prevent the conformational change in the N terminus of Bax, which is known to be necessary for translocation and activation; consistently, binding of an anti-Bax monoclonal was decreased after overexpression of Ku70. In addition, previous work has identified a cytosolic retention signal in the N terminus of Bax[13,14] and therefore it is possible that Ku70 recognizes this signal. Alternatively, perhaps no conformational change occurs at the N terminus, but the N-terminal epitope is instead masked by Ku70. Activation would then involve dissociation of Ku70 and subsequent binding by BH3-only proteins and possibly also by other Bax activators (Fig. 1a). However, more work will be needed to determine the precise site on Bax that Ku70 binds to. So far, the association of Ku70 with Bax has only been analysed in tissue culture cell lines. Activation of Bax seems to be a multistep process[3] and Bax may already be partially activated in cell lines, compared with intact tissues. Thus, it will be important to investigate the Bax–Ku70 interaction in tissues.

During the initiation of an apoptotic response, an inhibitor of Bax would need to be downregulated in some way. Interestingly, the levels of immunoreactive