Protein hijacking: Key proteins held captive against their will

Proteins travel to their appropriate intracellular homes by means of the targeting signals they carry. It now seems that a short, but important, list of key regulatory proteins are victims of protein hijacking: Bid, Bim, NF-κB, SREBP, and perhaps the intracellular portion of MUC1. These provide critical functions within a particular subcellular compartment, but are initially prevented from finding their way to this intracellular home. Only in response to specific physiological signals are these proteins released to find the site at which they act.

The signal hypothesis showed the intracellular location of proteins is dictated by targeting information within a protein's sequence (Blobel, 2000) and holds true for protein targeting to all subcellular locations (Schatz and Dobberstein, 1996). The efficiency and fidelity of protein targeting pathways are exquisite, provided the targeting sequence is openly displayed during, or very soon after, synthesis of the protein.

It is now becoming apparent that protein targeting, like so many intracellular processes, is subject to regulation. Proteins in transit to their intended intracellular destination can be hijacked and sequestered in a distinct subcellular compartment. In the cases we know of so far, only in response to a specific signal is the protein liberated to find its way to its site of action within the cell. The report in this issue of *Cancer Cell* by Kufe and coworkers on the intracellular portion of MUC1 (Ren et al., 2004) suggests it might join the list of protein hijack victims that include apoptosis regulators of the Bcl-2 family (Bim and Bmf) and transcriptional activators SREBP and NF-κB (Figure 1).

Normal cellular homeostasis is maintained by a balance in cell proliferation and apoptosis; disturbances in this balance directly contribute to cancer. Members of the Bcl-2 family of proteins are key regulators of apoptosis, and most of them carry C-terminal hydrophobic sequences capable of targeting them to membranes. However, some pro-apoptotic family members, such as Bim and Bmf, are sequestered away from their site of action at the mitochondrial outer membrane through interactions with cytoskeletal proteins: Bim with the microtubular dynein motor and Bmf with the myosin V motor (Puthalakath et al., 1999, 2001). Damage signals trigger their release and rapid translocation to the mitochondrial surface, where Bim and Bmf neutralize the prosurvival activity of other Bcl-2 family members, precipitating cell death.

The active forms of NF-κB and SREBP are potent transcriptional activators: NF-κB activates genes that regulate immune responses, cell division, and programmed cell death, whereas SREBP activates genes encoding enzymes of the cholesterol and fatty acid biosynthetic pathways. Each protein has a localization signal that could mediate targeting to the nucleus. However, newly translated NF-κB is hijacked in the cytoplasm by an interaction with the IκB family of repressor proteins. The IκB proteins act by covering the nuclear localization signal within NF-κB, thereby preventing its recognition (Ghosh and Karin, 2002). Exposure of cells to extracellular stimuli, such as ionizing radiation or cytokines, results in IκB-kinase activation, with subsequent phosphorylation and proteolysis of the IκB captor. This exposes the nuclear localization sequence of NF-κB and allows its entry into the nucleus to transactivate the expression of target

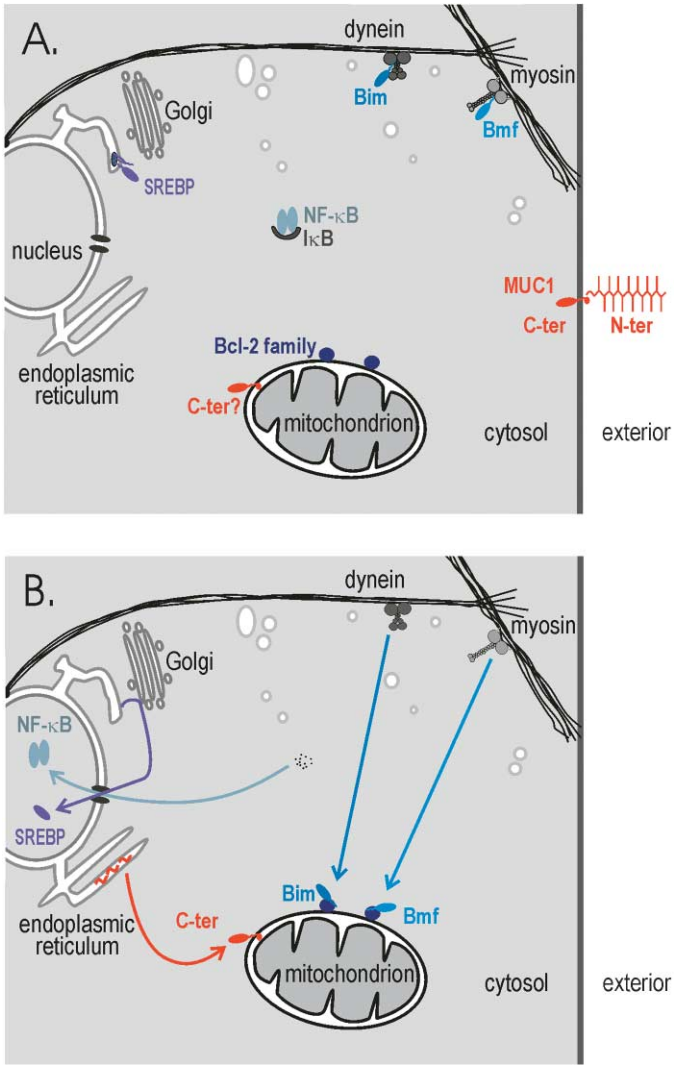

Figure 1. Release of hijacked proteins

A: Prior to external stimuli being applied, Bim and Bmf are held as components of cytoskeletal motor complexes, the NF-κB:IκB complex is cytosolic, SREBP is integrated and maintained in the endoplasmic reticulum, and the C-ter domain of MUC1 is associated with the heavily glycosylated N-ter domain at the cell surface. Some C-ter domain of MUC1 might also be associated with mitochondria.

B: After appropriate stimulation, Bim and Bmf can be released to target mitochondria, NF-κB released to the nucleus, and SREBP released for transit via the Golgi and final release for translocation into the nucleus. By some means, either retrograde translocation from the endoplasmic reticulum (shown), release from the plasma membrane, or expression from a novel variant mRNA, the C-ter domain of MUC1 is targeted to mitochondria.

genes. The importance of proper regulation of NF-κB activity for normal cellular physiology is underscored by the fact that constitutively active nuclear NF-κB, often due to hyperinduction of the NF-κB activation pathway and in some instances defective IκB activity or levels, contributes to the development and malignancy of numerous human carcinomas (Karin et al., 2002).

An even more complex scenario sequesters SREBP from the nucleus (Yang et al., 2002). The activation domain of SREBP is linked to a signal sequence that targets the protein to be cotranslationally integrated into the endoplasmic reticulum, where it interacts with a sterol-sensing protein, SCAP. SCAP collaborates with a retention factor to hold the SREBP-SCAP complex in the endoplasmic reticulum. When sterol levels in the cell are low, the retention factor releases SREBP-SCAP to escape to the Golgi, where proteolytic processing releases the activation domain of SREBP, which enters the nucleus to exert its function in activation of gene expression.

MUC1 is a cell surface oncoprotein, normally expressed on secretory epithelial cells and highly overexpressed in several human carcinomas. It is synthesized as a single polypeptide with a signal sequence that sends the protein into the endoplasmic reticulum. After processing to an N-ter extracellular fragment and C-ter transmembrane fragment, glycosylation of the extracellular domain allows MUC1 transport to the cell surface (Figure 1). Ren et al. show that treatment of cells with heregulin, a growth factor that signals via the ErbB pathway, induces mitochondrial targeting of MUC1 C-ter. Expression of MUC1 inhibits activation of apoptosis by genotoxic agents, and therefore, the consequence of sending MUC1 to mitochondria might be to inhibit the prodeath activity of other mitochondrial proteins. As proposed, this could be physiologically crucial: damage to the epithelium causes activation of the heregulin/ErbB pathway, inducing cell division for an effective replacement of damaged cells. Concomitant targeting of MUC1 C-ter to mitochondria might attenuate apoptosis during this stress response. Inhibition of apoptosis by MUC1 could have an important implication in its function in tumor physiology: constitutive mitochondrial localization of MUC1 C-ter observed in cancer cell lines (see below) might protect carcinomas from the apoptotic response following treatment with genotoxic agents.

So how and when is MUC1 C-ter targeted to mitochondria? MUC1 C-ter might go to mitochondria constitutively, since Ren et al. observe it to some extent as mitochondrial even in the absence of heregulin treatment. If this proves to be the case, MUC1 could be yet another example of proteins directly targeted to dual locations, with similar or different functions in each compartment (Silva-Filho, 2003). However, the heregulin-independent mitochondrial localization may be a result of either a more active ErbB pathway or higher MUC1 expression levels in cancer cell lines. MUC1 could then be another hijacked protein, restricted from mitochondria prior to a physiological signal.

How mitochondrial targeting of MUC1 C-ter is achieved is currently not clear, but there are several possibilities. It could be released directly from the plasma membrane, expressed from an alternative mRNA, or retargeted at the level of the endoplasmic reticulum. Retargeting from the endoplasmic reticulum could be a consequence of activation of a MAP kinase pathway caused by binding of heregulin to the ErbB receptor, since it signals the unfolded polypeptide response and cessation of glycosylation that would lead to retrograde translocation of proteins like MUC1 C-ter. The newly liberated C-ter fragment would be in the cytosol, but with an exposed hydrophobic (formerly transmembrane segment) available for binding to molecular chaperones that deliver unfolded proteins to mitochondria (Voos, 2003).

Far from some diabolical intent, it seems the hijackers and their captive proteins serve as sensors for specific stimuli. Since protein targeting is a rapid process that can be completed in the absence of ongoing transcription or translation, the targeting information in the hijacked protein enables a response to stimuli that might shut down other cellular activities. In the case of MUC1 C-ter targeting, interesting questions are now raised over how and from where the protein is liberated, what exactly it does once targeted to mitochondria, and whether it acts at the surface or from deep within the organelle. The work by Ren et al. on the function of MUC1 in resistance to genotoxic agents sets the stage for a wealth of further knowledge.

Ana Traven,[1] David C.S. Huang,[2] and Trevor Lithgow[3,*]

[1]St. Vincent's Institute of Medical Research
Fitzroy 3065
[2]The Walter and Eliza Hall Institute of Medical Research
Parkville 3050
[3]Department of Biochemistry and Molecular Biology
University of Melbourne
Parkville 3010
Australia
*E-mail: t.lithgow@unimelb.edu.au

Selected reading

Blobel, G. (2000). Chembiochem *1*, 86–102.

Ghosh, S., and Karin, M. (2002). Cell *109* (*Suppl*), S81–S96.

Karin, M., Cao, Y., Greten, F.R., and Li, Z.-W. (2002). Nat. Rev. Cancer *2*, 301–310.

Puthalakath, H., Huang, D.C., O'Reilly, L.A., King, S.M., and Strasser, A. (1999). Mol. Cell *3*, 287–296.

Puthalakath, H., Villunger, A., O'Reilly, L.A., Beaumont, J.G., Coultas, L., Cheney, R.E., Huang, D.C., and Strasser, A. (2001). Science *293*, 1829–1832.

Ren, J., Agata, N., Chen, D., Li, Y., Yu, W.-h., Huang, L., Raina, D., Chen, W., Kharbanda, S., and Kufe, D. (2004). Cancer Cell *5*, this issue.

Schatz, G., and Dobberstein, B. (1996). Science *271*, 1519–1526.

Silva-Filho, M.C. (2003). Curr. Opin. Plant Biol. *6*, 589–595.

Voos, W. (2003). Mol. Cell *11*, 1–3.

Yang, T., Espenshade, P.J., Wright, M.E., Yabe, D., Gong, Y., Aebersold, R., Goldstein, J.L., and Brown, M.S. (2002). Cell *110*, 489–500.