

## **Regulation of Function of the Menkes copper transporting P-type ATPase – role in health and disease**

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The Menkes (MNK, ATP7A) transmembrane copper-transporting P-type ATPase has a pivotal role in copper homeostasis. It is involved in systemic copper absorption, transport of Cu across the blood-brain barrier, important processes in the brain, transfer of Cu to Cu-dependent enzymes in the cellular secretory pathway, and in detoxification of copper. Mutations in the ATP7A gene lead to Menkes disease which is a severe and usually lethal Cu deficiency disorder. The MNK protein has been implicated in cis-platin resistance of tumours. MNK function is essentially regulated by its sub-cellular localisation. At basal Cu levels it is localised in the trans-Golgi network (TGN) where it delivers Cu to several cuproenzymes, whilst at elevated Cu it undergoes vesicular trafficking to the plasma membrane (PM) where it catalyses the efflux of excess Cu from the cell.

We are using cultured polarised MDCK cells to investigate the localisation and trafficking of MNK. We found that MNK is targeted to the basolateral membrane consistent with its role in systemic Cu absorption (gut) and reabsorption (kidney). Through *in vitro* mutagenesis we have identified a dileucine motif involved in targeting to the basolateral membrane and a PDZ target motif which appears to be involved in retention at this surface in elevated copper.

Kinase phosphorylation of MNK stimulated by elevated Cu is another level of regulation and this may be part of a signalling process that regulates localisation and trafficking in response to changes in Cu concentration. We have used mass spectrometry to identify phosphopeptides and are in the process of identifying the phosphorylated serine residues.

For further analysis of structure-function, we have purified MNK and reconstituted active enzyme in proteoliposomes. The data with purified enzyme suggests no requirement for other cofactors for catalytic activity.

In order to gain an insight into the role of MNK in development, we have identified the *Drosophila* homologue, DmATP7, and studied its expression at various stages of development. We have demonstrated specific expression patterns in early development and shown the essentiality of DmATP7 for completion for embryogenesis, early larval growth and development, neuronal function, and pigmentation.