S.14 INTRACELLULAR SORTING AND RECYCLING

S14.1
Mannose-6-Phosphate Receptors and Lysosomes

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In mammalian cells newly synthesized lysosomal proteins are targeted to lysosomes by two different mechanisms.

Mannose-6-phosphate residues are transferred to soluble lysosomal hydrolases during their passage through the Golgi-complex. In the Trans-Golgi-Network specific receptors recognize these carbohydrate markers. The receptor ligand complexes are sorted into clathrin coated vesicles which are fused with elements of the endocytotic pathway. Due to the acidic pH within the endosomes the lysosomal hydrolases dissociate from the receptors and are transported to lysosomes via an as yet unknown mechanism. Processing of the polypeptide and carbohydrate chains of the lysosomal proteins takes place on their way to and within lysosomes. The receptors recycle to the Golgi for another round of transport or to the plasma membrane. At the cell surface the endocytosis of lysosomal enzymes can be mediated. In the internalization of the receptors from the plasma membrane cytosolic proteins are involved which recognize signals in the cytoplasmic domains of the receptors. They mediate the concentration of the receptors in clathrin-coated pits.

Transport of membrane proteins depends on the signals that reside within their cytoplasmic tail. This is exemplified for the lysosomal acid phosphatase which is synthesized and transported as an integral membrane protein to lysosomes via the plasma membrane. The cytoplasmic proteins mediate the sorting at the TGN and the plasma membrane have distinct structural requirements for binding. Among the cytoplasmic receptors recognizing sorting signals within the cytoplasmic tails of lysosomal membrane glycoproteins and mannose-6-phosphate receptors the HAl and HA2 adaptors have been identified.

S14.2
Topography of Posttranslational Modifications in Yeast and Mammals

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Glycosylation, sulfation and phosphorylation of proteins occurs in the lumen of the Golgi apparatus. The membrane of this organelle contains carrier proteins which mediate the transport of cytosolic nucleotide derivatives into the lumen in a coupled exchange with the corresponding nucleoside monophosphate, an antiporter. A *S. cerevisaie* strain was constructed with a gene disruption in the Golgi GDPase. This strain was unable to synthesize *in vivo* GMP, the required antiporter for GDP-mannose transport. Analyses of proteins and mannosylinositol-containing sphingolipids showed that Golgi mannosylation was severely inhibited *in vivo* demonstrating that the antiporter mechanism occurs *in vivo*.[1]

We have also been studying the topography and mechanism of sulfation of heparan sulfate. The rat liver *N*-heparan sulfate sulfotransferase has been cloned[2] and found to catalyze two activities in glycosaminoglycans: *N*-acetylglucosamine deacetylation and *N*-sulfation.[3] *In vivo* expression of a cDNA encoding the enzymes led to increased sulfation of heparan sulfate strongly suggesting they catalyze the biosynthesis of heparan sulfate *in vivo*. We have also purified the PAPS transporter of the Golgi membrane 30,000 fold over homogenate.

1. Abeijon, C., Yanagisawa, K., Mandon, E., Hausler, A., Moremen, K., Hirschberg, C. B. and Robbins, P. W. (1993) submitted "Guanosine Diphosphatase is Required for Protein and Sphingolipid Glycosylation in the Golgi Lumen of *Saccharomyces Cerevisiae*."
2. Hashimoto, Y., Orellana, A. and Hirschberg, C. B. (1992) *J. Biol. Chem.* **267**, 15744–15750 "Molecular Cloning and Expression of Rat Liver *N*-heparan Sulfate Sulfotransferase."
3. Wei, Z., Swiedler, S., Ishihara, M., Orellana, A. and Hirschberg, C.B. (1993) *Proc. Natl. Acad. Sci. USA*, *in press*, "A Single Protein Catalyzes Both *N*-deacetylation and *N*-sulfation During the Biosynthesis of Heparan Sulfate."

S14.3
Sorting and Localisation of Glycosyltransferases to the Golgi Complex

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The signals reponsible for the localisation of two resident Golgi glycosyltransferases, namely β 1,4 galactosyltransferase (Gal T) and *N*-acetylglucosaminyltransferase I (GlcNAc TI) have been investigated. These membrane enzymes reside in the *trans* and *medial* Golgi compartments, respectively. Like other Golgi glycosyltransferases, Gal T and GlcNAc TI are membrane bound proteins with an N_{in}/C_{out} orientation, containing a short cytoplasmic domain, a dual signal/anchor domain, a short lumenal stem domain and a large lumenal catalytic domain. Species-specific antibodies have been generated to bovine Gal T and rabbit GlcNAc TI, and the expression and localisation of the enzymes have been examined in COS-1 cells and murine L cells transfected with cDNA constructs. Using these transfection systems we have explored the molecular basis of the sorting and retention of Gal T and GlcNAc TI to the Golgi complex. From the intracellular localisation of deletion mutants and of hybrid constructs we conclude that a signal contained within the transmembrane domain and flanking residues of the glycosyltransferases specifies compartment-specific Golgi localisation[1,2].

We have also developed a biochemical assay to quantitate Golgi retention of newly synthesised GalT molecules based on post-translational modifications which occur in the *trans*-Golgi network. Molecules retained within the Golgi stack (non-sialylated) can be distinguished from molecules transported through the *trans*-Golgi network to the cell surface (sialylated). This modification can be employed to monitor the active retention of newly synthesised molecules