proteases. The fragments were separated by SDS PAGE followed by Western blot analyses. The results indicate, that several fragments are shared by the fusion protein and glucosidase II. (ii) proteolytic fragments of glucosidase II were microsequenced. Oligonucleotide primers were synthesized according to these protein sequences and used for PCR amplification of DNA fragments coding for glucosidase II. Pig liver mRNA was prepared as a template. The amplified DNA fragments will be used for screening the λgt11 library. Combination of these two approaches should yield a full length nucleotide sequence information.

S2.25

Isolation of a Mouse cDNA Encoding a Processing Mannosidase

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Mammalian cells contain several α-mannosidases involved in oligosaccharide processing from $Man_9GlcNAc_2$ to $Man_5GlcNAc_2$, whereas in *S. cerevisiae,* only one processing α-mannosidase converts $Man_9GlcNAc_2$ to $Man_8GlcNAc_2$ in the ER. The deduced amino acid sequence of the yeast α-mannosidase gene (1) is homologous to that of cDNAs from rabbit liver and mouse 3T3 cells encoding an a-mannosidase which transforms $Man_9GlcNAc_2$ to $Man_5GlcNAc_2$ (2). Using regions of identity between the mammalian and yeast enzymes, primers were designed for PCR with mouse liver cDNA as template. A novel PCR product was isolated, and evidence was obtained for the existence of two different yet homologous mouse a-mannosidase genes (3). Using the novel PCR product as a probe, cDNA clones were isolated from a 3T3 cDNA library. The sequence of two clones shows the presence of an ORF encoding a type II membrane protein of about 73 kDa. Epitope tagging and transient expression of cDNA in COS cells followed by immunofluorescence indicates that the mannosidase is primarily localized to a perinuclear region corresponding either to the Golgi or to the intermediate compartment between ER and Golgi, with some cells also exhibiting a reticular staining in the cytoplasm.

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(2) Moremen *et al.* (1990) *Glycoconjugate J.* **7**:401.
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S2.26

Cloning of a Murine Homolog of the *Dictyostelium Disciodeum* Lysosomal α-Mannosidase

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The lysosomal α-mannosidase is a broad specificity hydrolase involved in the catabolism of mannose-containing glycoconjugates in the lysosomes of a wide range of organisms from slime molds to mammals. Although the mammalian enzyme has been purified from several sources, the cDNA encoding this enzyme has been cloned only from the cellular slime mold *Dictyostelium disciodeum* [1]. A protein sequence comparison between the slime mold enzyme and the murine Golgi α-mannosidase II [2] revealed an extensive sequence similarity despite a significant difference in substrate specificity between the two enzymes. In an attempt to clone the mammalian lysosomal α-mannosidase, we used the regions of protein sequence similarity between these two hydrolases to design degenerate oligonucleotide primers in order to identify additional related sequences from a murine cDNA library by PCR amplification. The amplification yielded two products, one of which was identified by restriction mapping as being the previously cloned Golgi α-mannosidase II. The other product was subcloned and sequenced. The nucleotide sequence and peptide translation indicated that the second amplification product was distinct from the processing enzyme: it was found to be a 620 bp continuous open reading frame bracketed by the oligonucleotide primer sequences used for its amplification. Comparison of the amplimer translation with the sequences of the *D. discoideum* α-lysosomal mannosidase and the murine Golgi α-mannosidase II indicated that it exhibited much greater similarity to the slime mold enzyme. Northern blots using RNA derived from a variety of rat tissues and the amplimer as a probe indicated a message size and differential tissue expression distinct from that of Golgi α-mannosidase II. Work is currently in progress to isolate the full-length cDNA for molecular and biochemical studies on the catabolic α-mannosidases in relation to mammalian-mannosidosis.

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(2) Moremen, K. W. and Robbins, P. W. (1991) *J. Cell Biol.* **115**, 1521–1534.

S2.27

Cloning of Man_9-Mannosidase from Human Kidney

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Man_9-mannosidase, an ER-resident (glyco)protein involved in the 'early' processing pathway of *N*-linked oligosaccharides, was recently purified and characterized from crude pig liver microsomes (Bause *et al.* (1992) *Eur. J. Biochem.* 208, 451-457). The enzyme differs in its substrate specificity from other α1,2-mannosidases in that it cleaves highly selectively three of the four α1,2-linked mannose residues from Man_9-oligosaccharides, generating a distinct Man_6-isomer. In order to understand the biology of this processing enzyme, we decided to clone its cDNA. We have isolated several Man_9-mannosidase-specific clones from a human kidney cDNA library in lambda gt 10, from which a 3250 bp cDNA could be reconstructed. This 3250 bp construct contains, in a single open reading frame, a 1875 bp region encoding 625 amino acids of the Man_9-mannosidase protein. The coding region containes the amino acid sequence of several BrCN peptides previously identified in pig liver Man_9-mannosidase, verifying the authenticity of the cDNA construct and, furthermore, documenting a surprisingly high degree of sequence homology between the pig liver and human kidney enzyme. The molecular mass of the Man_9-mannosidase protein calculated from its cDNA (73 kDa), is identical with that of a protein species identified in a microsomal fraction of human kidney tumor cells by immunoblotting. The amino acid sequence of