The Ph.D. thesis is focused on lysosomal storage disorders (LSD) with impaired lysosomal metabolism of glycans, namely on Fabry, Schindler, Pompe diseases and muccopolysaccharidosis (MPS) type IIIC. We hypothesized that the worm, which has active glycan metabolism, will have orthologs corresponding to human enzymes deficient in the
above disorders and could be a suitable model for study of cellular pathology of these LSDs.
The protein families of glycosidases which are deficient in the first three disorders are well
characterised while the protein deficient in MPS IIIC was unknown and its enzymatic action
was only partially characterized. The gene deficient in MPS IIIC was recently identified in our
lab (publication 2) and, as is shown below, it belongs to the lysosomal proteins that have no
apparent ortholog in the worm.
The main task of the thesis was to find and characterize the *C. elegans* orthologs of human α-galactosidase (α-GAL), α-N-acetylgalactosaminidase (α-NAGA), acid α-glucosidase
(GAA) and heparin acetyl-coenzyme A:α-glucosaminide N-acetyltransferase (HGSNAT) whose deficit leads to Fabry, Schindler, Pompe and MPS IIIC diseases, respectively, and to study their biological functions in *C. elegans* by RNA-mediated interference (RNAi). Additional aims were to perform basic bioinformatic analysis and characterize tissue and cellular distribution of HGSNAT mainly at microscopical level.
We found a single *C. elegans* ortholog GANA-1 of both human α-GAL and α-NAGA proteins which are presumed to share a common ancestor. Biochemical, RNAi, GFP expression and bioinformatic analyses confirmed our supposition that GANA-1 has dual
enzymatic activity and is localized in the acidic cellular compartment and showed that *gana-1*
probably developed from the hypothetical ancestral gene before the duplication event which
led to formation of two homologous animal genes: α-Gal and α-Naga.
We found four potential *C. elegans* orthologs (AAGR-1-4) of human GAA. We analysed these orthologs by combination of biochemical, cell biological and bioinformatic
approaches and determined that AAGR-1 and -2 have predominantly acidic and AAGR-3 and
-4 neutral pH optimum and that AAGR-2 is prevalent contributor to the total acidic and
AAGR-3 to the total neutral GAA activity. We found out that AAGR-1 and -2 are localised in
membrane-bound cellular compartments and that AAGR-2 is inhibited by acarbose, in contrary to AAGR-1 which is not inhibited. These results were further supported by the

analysis of *aagr-1* deletion mutant. Based on these finding we consider AAGR-1 the most
probable true ortholog of human GAA.
We found out that RNAi did not provide sufficient decrease of glycosidases activities which is necessary for development of lysosomal storage and we assumed that relevant deletion mutant could be suitable models.
We verified the sequence of HGSNAT and found that cDNA is 4.5 kb long, is composed of 18 exons and is expressed in various human tissues. Basic bioinformatic analyses
showed that HGSNAT contains N-terminal signal peptide, 11 predicted transmembrane
domains and 4 potencial N-glycosylation sites. We found out that HGSNAT does not share
sequence homology with any known prokaryotic or eukaryotic N-acetyltransferase except its
mammalian orthologs. Analysis of the cellular distribution of HGSNAT revealed that HGSNAT is present only in a subpopulation of lysosomes and is localised in microdomains of
lysosomal membranes. Based on partially co-lokalisation of HGSNAT and sortilin we hypothesize that lysosomal sorting of HGSNAT could be sortilin dependent.