RNAi screening of human glycogene orthologs in the nematode Caenorhabditis elegans and the construction of the C. elegans glycogene database (CGGDB)

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Abstract of the thesis

The cell surfaces of living organisms including bacteria, archaea, yeasts, nematodes, insects, and mammals are covered with sugar chains. Their wide distribution and evolutionary conserved presence on cell surfaces strongly indicate that sugar chains are indispensable components of living organisms. Sugar chains are involved in pathogenic mechanisms of viral infection, bacterial infection, tumorigenesis, muscular dystrophy, and other diseases. Thus, sugar chains involved in pathogenic mechanisms could be potential targets of drugs, and the study of glycans (known as glycobiology) is becoming more and more important for pharmaceutical applications. Glycans are synthesized by successive reactions catalyzed by glycosyltransferases, sugar transporters, and glycan modifying enzymes such as sulfotransferases and sialyltransferases. Degradation of sugar chains is also important in homeostasis and the timely expression of glycans on cell surfaces. The term glycogene is defined as such genes involved in synthesis, degradation, transport, and modification of glycans in living organisms. The aim of this study is to identify human glycogene orthologs in the genome of *Caenorhabditis elegans* (*C. elegans*), and to examine the phenotypes when each glycogene is inhibited by RNAi experiment. With the experts of bioinformatics for glycobiology and glycogenomics at the AIST (National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan), I constructed a new database CGGDB (*C. elegans* glycogene database) and the database includes all the results of the thesis (excluding unpublished results) as well as all the known essential information on each glycogens collected from other databases (e.g., Wormbase and NEXTDB (*C. elegans* in situ hybridization database)).

C. elegans is a soil living nonpathogenic nematode, and it is widely used as a model organism for biological sciences. It is the first multicellular organism whose genome DNA sequence is completely determined, and it has been playing leading roles in the recent development of biological sciences. The use of the nematode as a model organism for studying mechanisms of pathogenesis is becoming very popular recently. As described above, various diseases are caused by abnormal/normal sugar chains, thus identifying human glycogene orthologs in the genome of *C. elegans* and examining the functions of the glycogenes could be very useful for the study of molecular mechanisms of various human glycodiseases.

In this study, I identified 195 possible human glycogene orthologs in the genome of

the nematode, and examined the RNAi phenotypes of most of the glycogenes. By using homology search (BLAST and PSI-BLAST search) and by using various databases, Prof. Hisashi Narimatsu's group picked up 108 possible orthologs of human glycogenes, and our group has been carrying out functional glycomics analyses based on their prediction. I added 87 new candidates of human glycogene orthologs based on analysis with PSI-BLAST followed by bidirectional best fit analysis. Ortholist (a *C. elegans* human ortholog database), Cazy database and recent literatures on human glycogenes are also used for the identification. Thus, I studied 195 *C. elegans* glycogenes orthologous to human glycogenes with RNAi and partly with deletion mutagenesis. Newly added 87 genes include various genes involved in GPI-anchor synthesis, and orthologs of newly found human glycogenes. For RNAi analyses of the selected 195 genes, reliability of RNAi constructs of the Ahringer RNAi library was checked, and the use of non-reliable strains of *E. coli* for the feeding RNAi (14 strains) were excluded. Thus, I carried out RNAi-screening of 181 glycogene orthologs. Special attention was paid on the germline development (germline mitosis, and meiosis) and early embryonic division. ER (endoplasmic reticulum)-stress phenotype (RNAi) was also monitored with the transgenic worm expressing GFP driven by BiP promoter. BiP is an ER-stress sensor of the nematode. Among the 181 genes examined, 38 glycogenes showed RNAi phenotypes, and the results are summarized in the thesis, and all the results are deposited in the CGGDB.

Inhibition of chondroitin synthesis resulted in abnormal oocyte formation, and inhibition of sulfotransferases (*hst-2* and *hst-3.1*) also resulted in abnormal oocyte formation. The results indicate that formation of glycosaminoglycan (GAG) is essential in the germline formation. Inhibition of some of the genes involved in N-glycosylation also resulted in similar abnormalities. N-glycosylation begins at the cytoplasmic side of the ER, and the lipid-liked oligosaccharide (LLO) synthesis is the first step of the N-glycosylation which begins at the cytoplasmic side of the ER. Inhibition of genes involved in the earlier steps of LLO synthesis (*alg-7*, *alg-13*, *alg-14*, *alg-1*, *alg-2*, alg-5, *dpm-1*, and *alg-11* orthologs which are active at the cytoplasmic side of the ER) resulted in abnormal oocyte formation. The ER stress phenotype and body size small phenotype were detected in almost all of the LLO synthesis genes active at the cytoplasmic side of the ER. The LLOs synthesized at the cytoplasmic side flip to the ER lumen side, and they are processed with the gene products coded by *alg-3*, *alg-9*, *alg-12*, *alg-6*, *alg-8* and *alg-10* orthologs. All these *alg* gene orthologs active at the ER-lumen side did not show the RNAi phenotypes. Genes involved in the attachment of LLO to asparagine residue of a protein (oligosaccharyltransferase complex genes) showed severe phenotypes similar to the

RNAi phenotypes of the cytoplasmic *alg* genes.

The *alg* genes and *dpm1* gene acting at the ER-cytoplasmic or the ER-lumen side are responsible genes for human congenital disorder of glycosylation (CDG) type 1. Tunicamycin which inhibits the first step of LLO synthesis catalyzed by *alg7* gene product has been extensively used for the study of CDG type 1. Instead of using the drug, RNAi of the *C. elegans* cytoplasmic *alg* genes resulted in severe and repeatable inhibition phenotypes similar to the ones found in the human CDG. Thus, this study shows for the first time that *C. elegans* could be a potential and useful model organism for the study of CDG type 1.

A shorter and edited version of the thesis is published in the journal Glycobiology (Glycobiology (2014) doi: 10.1093/glycob/cwu080). All the data in the thesis (excluding the results to be published separately) can be found in the paper, its accompanying supplemental data, and in our database CGGDB.