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Grant Title: Study on the roles of glycogenes involved in cell division of *C. elegans*

Abstract

C. elegans is an ideal model organism for studying functions of glycoconjugates in development, differentiation, and morphogenesis. The inhibition of gene function via RNAi and deletion mutagenesis is quick and easy, and abnormal phenotypes can be observed in vivo by using time-lapse microscopy and by using various transgenic worms expressing fluorescent protein-tagged proteins. By using this model organism, we have been studying functional roles of glycogenes. We previously reported that chondroitin synthesis, GlcCer synthesis and GPI-anchor synthesis are indispensable for oogenesis and/or early embryonic cell division.

To identify glycogenes involved in cell cycle progression, we listed human glycogene orthologs in the worm genome by bioinformatics, and carried out RNAi of all the selected human glycogene orthologs. Since oocyte formation and early embryonic cell division can be observed in vivo easily, we paid special attention to the germline phenotypes and early embryonic cell division. Besides abnormalities in oogenesis and early embryonic cell division, we also examined whether unfolded protein response (UPR) is induced when each glycogene is knocked down with RNAi. For this purpose, worms expressing P*hst-4*::*gfp* gene product (*GFP* gene linked to the promoter sequence of *hsp-4*, a worm *BiP* ortholog) were RNAi-treated and the expression of the GFP was monitored by using COPAS^{TM} Biosort (a laser fluorescence worm sorter).

By knocking down all the 156 glycogenes selected, we confirmed most of the previously reported RNAi phenotypes, and found novel RNAi-phenotypes. RNAi of chondroitin synthesis resulted in abnormalities of early embryonic cell division (*sqv-5* and *mig-22* RNAi) while oogenesis was severely affected in *mig-22* RNAi-treated animals. RNAi of sulfotransferases that are essential for neuronal wiring (*hst-2* and *hst-6*) also resulted in malformation of the germline. RNAi of *hst-3.1* resulted in abnormal oogenesis. The results indicate that sulfotransferases are essential for oogenesis. RNAi of glycogenes involved in N-glycosylation were also extensively tested. RNAi of genes involved in the early phase of LLO (lipid-linked oligosaccharides) synthesis resulted in severe germline phenotypes in addition to UPR, while RNAi of genes involved in the later phase of LLO synthesis resulted in no abnormalities. Inhibition of oligosaccharyltransferase genes (*stt-3*, *dad-1*, *ostb-1*, *ribo-1* etc.) resulted in severe germline phenotypes and UPR. The results strongly indicate that N-glycosylation (LLO synthesis and attachment of the LLO to proteins) is essential for normal oogenesis to occur. Phenotypes observed for the 156 tested glycogenes are compiled in our newly constructed database *C. elegans* GlycoGene DataBase (CGGDB) at AIST, Tsukuba, Japan. In addition to the results of our RNAi experiments, information on each *C. elegans* glycogene including DNA sequence, amino acid sequence, human orthologs, BLAST results, links to WormBase and in situ database, phenotypes observed (RNAi and gene variant), and references are concisely compiled. We hope our database is useful for the researchers who want to begin their analysis of any *C. elegans* glycogenes of their interest.