Worms found a sweet way to protect themselves from pathogens

Preview for "Jeong et al. (2020) Inhibition of the oligosaccharyl transferase in Caenorhabditis elegans that compromises ER proteostasis suppresses p38-dependent protection against pathogenic bacteria. PLoS Genetics, 16(3):e1008617"

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Glycoproteins play important roles in physiological and pathogenic processes.[1] The biosynthesis of N-glycans is initiated on the endoplasmic reticulum (ER) membrane and then in the ER lumen the synthesized N-glycans are transferred to the asparagine of the proteins with a conserved motif NXS/T, where X can be any amino acids except proline, which is catalyzed by the oligosaccharyl transferase (OST).[1] Previously, it is known that glycosylation of proteins is important for the activities of the immune system.[2] The immune defense of *Caenorhabditis elegans* (*C. elegans*) is highly related to protein homeostasis.[3] However, how does the aberrant glycosylation caused by OST deficiency impacts immune response is still unknown. *C. elegans* is an excellent animal model to study the genetic and molecular mechanisms of immune-response.[4] In *C. elegans*, the PMK-1/p38 pathway is required for resistance to bacterial infection.[5–7] After infection with pathogenic bacteria such as PA14, the reference strain of *Pseudomonas aeruginosa*, the PMK-1/p38 MAP kinase is induced in *C. elegans* and this signaling activates the downstream transcription factors, including the ATF-7, an ortholog of the human ATF2 and CREB5 that regulates defense response to Gram-negative bacterium and innate immune response; ELT-2, an ortholog of the human GATA4 and GATA5 that modulates the defense response to other organisms; and SKN-1, which controls ER unfolded protein response (UPR^{ER}).[8] During the protection process against PA14, the UPR^{ER} regulators including IRE-1 (an ortholog of human ERN1, a transmembrane serine/threonine protein kinase, exhibits endoribonuclease activity) and XBP-1 (X-box binding protein 1, an ortholog of human XBP1) are mobilized. In this paper, the authors investigated the mechanisms of how the OST complex for protein glycosylation impacts the PMK-1 signaling and UPR^{ER} to protect *C. elegans* from PA14 infection.

In this paper, the authors discovered that RNAi knockdown of several OST complex components decreases the survival of *C. elegans* on PA14 and started investigating the underlying molecular mechanisms. They first found that inactivation of the OST complex does not affect the lifespan of worms on pathogenic *E. coli* or *E. faecalis* but PA14, suggesting the OST complex modulates specifically the immune response against PA14 infection. To examine the OST substrates that are crucial for this response, they performed a small-scale two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) followed by glycoprotein staining. Then, via peptide mass fingerprinting they searched for glycoproteins whose levels are induced

by PA14 infection in an OST dependent manner. They found that one of the OST-dependent N-glycosylated proteins VIT-6, which is a vitellogenin protein that is expressed in the intestinal cells and gets imported to the eggs. VIT-6 was identified in a previous N-glycoproteomic study and in this new paper the authors further demonstrated that N-glycosylation of VIT-6 is essential for its secretion and accumulation in the eggs and also required for PA14 survival. Together, the authors discovered that the OST complex is required for PA14 immune response and they further demonstrated an efficient methodology to examine glycosylated proteins, which identified VIT-6 as an OST complex substrate and as essential for protection against PA14.

Next, the authors set out to find the OST complex dependent transcriptional changes induced by PA14 infection by performing RNA sequencing. They first noticed that knockdown of *stt-3*, an OST complex subunit, significantly alters transcriptome profile even without PA14 infection. Essentially, genes associated with UPR^{ER} and immune response are induced, whereas metabolic genes are significantly downregulated. They showed that knocking down several OST complex subunits induces the expression of *[hsp-4::GFP],* a UPR^{ER} reporter and increases the alternative splicing of *xbp-1* mRNA, another indicator of UPR^{ER}. On the other hand, their findings suggest that although induced, UPR^{ER} is not the major cause for increased susceptibility for PA14 infection in OST complex inactive worms. Finally, they compared their RNAseq data with previously published *C. elegans* transcriptome data and showed significant overlap between OST-dependent PA14 induced genes and the genes whose induction is dependent on ELT-2, PMK-1 and ATF-7, the set of key transcription factors acting downstream of PMK-1 immune response signaling. They further demonstrated that other OST complex components, and not just *stt-3*, are required for PMK-1 dependent gene induction in response to PA14-infection. In conclusion, they discovered that OST complex mediates ER homeostasis by maintaining UPR^{ER}, but protects against PA14 infection independent of UPR^{ER} signaling, rather by activating PMK-1 signaling.

In this study, Jeong *et al.* discovered an interesting link between organism-level immunity and N-glycosylation which was not commonly studied in *C. elegans* before. They found that the OST complex is required for immune response against pathogenic *P. aeruginosa*, PA14. However, many interesting questions remain. First of all, how does the OST complex substrate identified in this paper, VIT-6, protect against PA14 infection? The authors speculate that the lipid transport function of VIT-6 may be playing a role in this regulation or as a secreted factor it may be activating a signaling pathway involved in immune response. Some future studies can also examine whether the OST-dependent N-glycosylation is specific for VIT-6 or other vitellogenins are glycosylated as well. This brings us to the second question: What is the specificity of their methodology to detect glycosylated proteins? 2D-PAGE combined with glycosylated protein staining followed by peptide mass fingerprinting may not be as sensitive as mass spectrometry, especially in a screen-based manner. Therefore, it will be more compelling to investigate the OST substrates via mass spectrometry and detect more candidates relevant to the

immune response activation against PA14. The OST complex is involved in N-glycosylation of all proteins, thus there are likely several glycoprotein targets whose functions are important in UPR^{ER} and immune response in *C. elegans*. To more clearly and deeply answer the question of whether it is a non-canonical signal transduction function of the OST complex or it is indeed the specific downstream glycoproteins that contribute to these processes, it is essential to examine the OST targets in a more sensitive way. Finally, the third question is in addition to UPR^{ER}, are other stress responses activated upon inhibiting the OST complex? Examining those will significantly broaden the mechanistic characterizations of the OST complex and identify more of its N-glycosylated protein targets. Overall, Jeong *et al.* opened up a new venue for studying protein glycosylation in a whole organism setup, specifically in the context of immunity in this current paper and likely more studies will follow this lead to investigate further links between protein glycosylation and organism fitness.

References

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