Orphan enzyme cuts down on sugar

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The reclusive enzymes that catalyze proteolysis inside the cell membrane are among the most widespread in nature, yet most remain ‘orphans’ whose cellular functions are poorly understood. Now, Voss *et al* identify Golgi-resident glycosyltransferases and glycosidases as substrates for the presenilin-like protease SPPL3. Shedding of these glycan-modifying enzymes from the membrane down-regulates global protein N-glycosylation.

See also: **M Voss *et al*** (December 2014)

Proteolysis inside the membrane came to light through the studies of essential biological processes and disease states (as reviewed in Urban, 2013). Widespread genome sequencing revealed that the novel enzymes that catalyze this unusual reaction exist in large superfamilies in nearly all known organisms. Assigning functions to these other ‘orphan’ homologs, however, has turned out to be a slow process.

Among the first of such intramembrane proteases to be identified was presenilin, which, as the catalytic component of the γ -secretase complex, took center stage because of its involvement in the pathogenesis of Alzheimer’s disease (De Strooper & Gutierrez, 2015). Sequencing of the human genome revealed five additional presenilin-like proteins, one of which was named signal peptide peptidase (SPP), because it cleaved signal peptides after their removal from nascent proteins (Weihsen *et al*, 2002). SPP activity on signal peptides was soon found to play roles in the immune system and be hijacked by the hepatitis C virus as a processing enzyme for capsid assembly (Voss *et al*, 2013).

Inigorated by this wealth of new information, researchers set their sights on studying other signal peptide peptidase-like

(SPPL) enzymes, but clear functions have been slow to emerge and several remain functional orphans (Voss *et al*, 2013).

Perhaps one fewer orphan exists today. Through careful observation, Voss and colleagues noticed that the electrophoretic migration of a subunit of the γ -secretase complex was altered when analyzed from human cells overexpressing SPPL3 (Voss *et al*, 2014). Louis Pasteur forecasts that “in the fields of observation, chance favors only the prepared mind”: not content to ignore their chance observation as just a meaningless overexpression artifact, Voss and colleagues explored this further and found similar altered migration with other, unrelated membrane proteins, and the effect required SPPL3 protease activity. Armed with these general clues, they formulated a specific hypothesis: perhaps SPPL3 was affecting the sugar signature of membrane proteins by cleaving the enzymes responsible for modifying sugars.

Nearly all proteins that enter the endoplasmic reticulum (ER) are initially ‘stamped’ on select asparagine (N) residues with an elaborate branch of 14 sugars (Ferris *et al*, 2014). This N-glycosylation ‘entry stamp’ is further modified in the ER by different glycosidases that trim terminal sugars to reflect whether the protein has achieved the properly folded state (Ferris *et al*, 2014). Once folded, the protein is ready to be exported to the Golgi apparatus, where new enzymes elaborate the N-glycosylation signature further in complex ways. Ultimately, these customized sugar coatings help to determine where the client protein is taken in the cell, modulate its function and/or how long it lives (Bieberich, 2014).

Knowing that SPPL3 resides in the Golgi apparatus (Voss *et al*, 2013), the authors suspected that, if their hypothesis was correct, only the late ‘complex’ sugar modifications should be altered, and not the

early high-mannose or intermediate hybrid glycans. A combination of deglycosylating enzyme treatments, glycosylation inhibitors and a glycome analysis by LC-MS revealed this to be true and provided the next clue: are the enzymes responsible for these late modifications being cut by SPPL3?

Relative to presenilin, SPPL3 adopts an inverted topology and thus cuts membrane proteins of type II (N cytoplasmic) orientation (Friedmann *et al*, 2004). Several glycosyltransferases are known to be type II membrane proteins, with their transmembrane anchors lying close to their amino termini (Bieberich, 2014). Accordingly, the authors found that the key glycosyltransferase GnT-V, which provides a branching point in glycans, is reduced in cells overexpressing SPPL3. The ectodomain containing its glycosyltransferase active site was found concomitantly secreted into the culture media, and cleavage site mapping revealed the location of the liberating cut to be consistent with SPPL3 processing.

Interestingly, reducing GnT-V levels had a milder effect than overexpressing SPPL3, suggesting SPPL3 may also cleave additional glycan-modifying enzymes. Accordingly, the authors identified a further three glycan-modifying enzymes as substrates for SPPL3. Notably, this shedding down-regulates glycosylation, because the enzyme ectodomains retain activity but find themselves in a compartment devoid of the nucleotide or lipid-activated sugars that they rely upon as substrates for catalysis. This observation tied well with prior findings, because some glycosyltransferases had been enigmatically found to be secreted under certain conditions, yet the precise mechanisms remained uncertain.

But while the initial observations started from overexpression studies in cultured cells, the analysis could not stop there to be truly meaningful. The authors obtained

Figure 1. SPPL3 regulates cellular protein N-glycosylation.

'Lighter' SPPL3 expression (right) results in more active glycosyltransferases/glycosidases (GTs) on the Golgi membrane and thus more protein N-glycosylation (diagrammed as a red/white 'sugar coat' around the cell). 'Heavier' SPPL3 expression (left) sheds more GTs from the Golgi membrane, which results in less overall protein N-glycosylation.

SPPL3-deficient mice and examined glycosylation of several proteins in various tissues. Consistent with their *in vitro* observations, SPPL3 deficiency in mice resulted in broad hyperglycosylation and increased cellular levels of GnT-V in mouse embryonic fibroblasts.

Like many breakthroughs in science, the current study opens many more new and exciting questions. An attractive feature of the resulting model is that proteolytic cleavage can simultaneously regulate a whole battery of different glycan-modifying enzymes in a concerted, network manner. However, while the authors already succeeded in identifying no fewer than four shed glycan-modifying enzymes, the full repertoire of SPPL3 targets is not yet understood. In fact, it remains an open question whether SPPL3 is specialized for regulating N-glycan modifications, or whether it might also contribute to regulating O-linked sugars. Future proteomic studies to identify SPPL3-cut targets promise to complete the list.

Perhaps the most intriguing mystery that lingers is what role SPPL3 regulation plays. The key advantage of deploying proteases to regulate cell biology lies in their ability to

exert control rapidly in response to a particular condition. In contrast, transcriptional mechanisms, which are known to regulate the expression of glycan-modifying enzymes including GnT-V (Trinchera *et al*, 2014), mediate slower, long-term changes. However, what triggers SPPL3 to cleave glycosyltransferases and glycosidases is not understood, and neither is the ultimate effect of reducing the N-glycan profile of the client proteins on their functions or that of the cell.

Finally, congenital disorders of glycosylation (CDGs) are a vast array of inborn diseases in > 100 genes, most of which affect N-glycosylation. Gene identification in this area has accelerated to an amazing pace; in 2013, a new CDG gene was identified, on average, every 17 days (Freeze *et al*, 2014). With its prominent role in affecting N-glycan signatures of potentially hundreds of proteins, it is quite tempting to ponder whether SPPL3 might itself be one of the lingering unidentified CDGs. Moreover, SPPL3-deficient mice are not born in the expected Mendelian ratios, suggesting that additional developmental roles, either involving N-glycan regulation or completely different functions through as yet

unidentified substrates, may remain to be discovered for this intriguing protease.

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