UNFOLDED PROTEIN RESPONSE

Letting go of stress

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The unfolded protein response (UPR) pathway helps cells counteract stress that perturbs folding of proteins in the endoplasmic reticulum (ER). UPR resets proteostasis by lowering protein flux into the ER through translational downregulation or mRNA turnover and by elevating the expression of ER proteins that manage accumulated unfolded proteins. Reid *et al.* now report a new pathway that limits protein transit into the ER by dynamic relocalization of mRNA and ribosomes to the cytoplasm. Using thapsigargin and dithiothreitol as reagents to induce protein folding stress in cells, the authors assessed the location and activity of translation over time using ribosome profiling and RNA-seq. Their analysis suggests that early UPR focuses on lowering translational efficiency—particularly for the subset of ER-tethered polyribosomes that are translating mRNAs for membrane and secretory proteins—in a rapid process that involves selective release of these mRNA–ribosome complexes into the cytoplasm, where they may continue translation. This pathway is also reversible, as kinetic analysis revealed that this translating pool of mRNAs becomes relocalized to the ER membrane upon removal of folding stress. Though additional studies will be needed to identify the molecular interactions that regulate ribosome tethering and release at the ER, the current model provides a new mechanism for reducing protein flux stress at the ER during the early stages of UPR. *TLS*

CARBOHYDRATES

Cutting out starch

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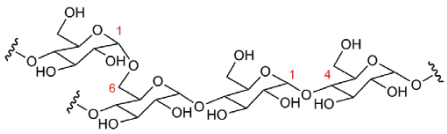

Polysaccharide monooxygenases (PMOs) are Cu-dependent enzymes that cleave carbohydrate polymers including chitin, cellulose and hemicellulose. The structures of these enzymes display several conserved elements, including a Cu-coordinating 'histidine brace'. However, their sequence homology is low, making discovery of new family members difficult. Vu *et al.* now use the signal peptide associated with these secreted proteins along with the strict requirement for an N-terminal histidine—part of the histidine brace—to search *Neurospora crassa* for putative PMOs. The authors found 21 such proteins whose sequences also contained the second residue of the histidine brace and a known active site motif. NCU08746 was notable in containing a C-terminal domain related to the family 20 carbohydrate-binding module (CBM20), also known as the starch-binding domain, and so was predicted to bind amylose, a substrate not yet represented among PMOs. *In vitro* characterization demonstrated that NCU08746 can cleave several starch substrates. This activity is dependent on oxygen and a reductant, a role that can be played by the cellobiose dehydrogenase known to pair with cellulose-degrading PMOs, though whether this partnership occurs *in vivo* remains unknown. The enzyme binds and is dependent for activity on a single copper atom, and spectroscopic data support a metal binding site consistent with those of other PMOs. These results provide an important expansion of the PMO family and offer a new starch-degrading enzyme for potential industrial applications. *CG*

TARGET IDENTIFICATION

NAD salvages neurons

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Despite the importance of protecting newborn and adult hippocampal neurons from death, there are no therapeutic strategies available. A multiyear screening approach aimed at identifying compounds that enhance hippocampal neurogenesis in adult mice identified a lead scaffold, P7C3. P7C3 analogs mitigate the normal turnover of newborn neurons. To determine the molecular target of P7C3, Wang *et al.* first developed a cell-based assay that reports on P7C3 protection from toxicity. The authors found that among the eight different toxins and hundreds of proneurogenic compounds they tested, only the P7C3 scaffold compounds protected cultured cells from apoptosis induced by doxorubicin (dox) and that this activity correlated with the proneurogenic activity of P7C3 in living mice. Using a photo-cross-linking approach and two-dimensional gel electrophoresis aided by a clickable version of P7C3, the authors identified NAMPT as the compound's target. NAMPT is the rate-limiting enzyme in the NAD salvage pathway, and dox depletes cellular NAD levels, so discovering NAMPT's role as the P7C3 target could explain its ability to protect against dox-mediated toxicity if it is able to enhance NAMPT activity. Indeed, the authors found that P7C3 could activate NAMPT and replenish NAD levels depleted by dox. There was a striking correlation between the ability of 30 P7C3 variants to activate NAMPT *in vitro*, protect neurons from dox toxicity, compete with photo-cross-linking by an active P7C3 variant and restore NAD levels in dox-treated cells, validating NAMPT as the efficacy target and implicating it in regulating neurogenesis. *MB*

PROTEIN TURNOVER

Mitochondrial immaturity

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Mitochondrial proteins synthesized in the cytosol contain a cleavable N-terminal presequence that directs them to their destination. Upon arrival in the mitochondrion, the presequence peptide is removed by the processing peptidase MPP and subsequently degraded by the matrix peptidasome Cym1 (also known as PreP). In the mitochondria of yeast *cym1* mutants, Mossmann *et al.* detected the accumulation of proteins containing the N-terminal presequence, suggesting that MPP activity was dependent on peptide degradation. The unprocessed proteins were identified as being required for mitochondrial processes such as ATP synthesis and respiration, which correlated with the defects observed in *cym1* mutants, such as increased reactive oxygen species and decreased membrane potential. Interestingly, Cym1 was previously shown to degrade the amyloid-β peptide (Aβ), and the mitochondrial defects observed in *cym1* mutants resembled features observed in Alzheimer's disease (AD) patients. The introduction of Aβ produced the same feedback effects on MPP activity as *cym1* mutants with the accumulation of unprocessed mitochondrial proteins. Finally, the maturation of mitochondrial preproteins was also inhibited in mouse and human AD brain samples, suggesting a potentially conserved mechanism to explain the effects of Aβ peptide on mitochondrial function. *GM*

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