

NEUROBIOLOGY

Salt and sensibility

Science 345, 313–317 (2014)

In *Caenorhabditis elegans*, the insulin-PI3K signaling system controls nervous system function, including associative learning. In a basic assay of associative learning, worms are attracted to specific concentrations of salt if they have been previously exposed to it during a meal, but they avoid those concentrations previously associated with concomitant starvation. To understand the regulation and function of the insulin-PI3K system in this *C. elegans* taste-avoidance learning system, Ohno *et al.* first looked at mutants of various components of the signaling pathway, including that of the insulin receptor, DAF-2. They also looked at mutants in *casy-1*, which encodes a cadherin superfamily membrane protein implicated in learning. Both sets of mutants displayed impaired associative learning. Using RT-PCR, the authors found a *daf-2* splice variant, *daf-2c*, which represents the insertion of an additional exon within *daf-2a* that is predicted to lengthen the DAF-2a protein's extracellular region. *daf-2c* (but not *daf-2a*) could complement the defect in taste-avoidance learning as well as the increased salt-induced synaptic release associated with *daf-2* mutants. DAF-2a localized to the cell body, whereas DAF-2c was localized in neuronal axons in the salt-sensing gustatory neuron ASER, and this localization was dependent on CASY-1 and was increased upon starvation. The authors also found that CASY-1 acts as a direct link between DAF-2c and kinesin, playing a part in axonal localization of DAF-2c. Finally, the authors found that ERK/MAPK pathway components may transmit food signals through CASY-1 and kinesin to regulate axonal localization and therefore the function of DAF-2c.

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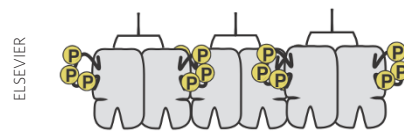
expelling plasmids from the cell. To bypass bacterial pushback, Zeng *et al.* co-opt the rarely used AGG codon to insert their residues of interest. Indeed, using a plasmid encoding the pyrrolysine tRNA and synthetase along with GFP as a test protein, the authors were able to incorporate three amino acids at yields up to 92% of the total GFP produced, with no obvious toxicity to the cells.

CG

ER STRESS

To live or let die

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Unfolded proteins in the endoplasmic reticulum (ER) trigger an unfolded protein response (UPR) to ensure the restoration of protein homeostasis. One of the signaling outputs for the UPR, IRE1 α , senses unfolded proteins through its luminal domain, triggering IRE1 α oligomerization and trans-autophosphorylation of the kinase domain, thus activating the adjacent RNase domain, which cleaves the XBP1 transcription factor. This process, which results in an adaptive UPR, differs from a terminal UPR, which occurs under high or unresolvable protein stress. In a terminal UPR, IRE1 α cleaves a larger number of ER mRNA targets (called extra-XBP1 cleavage) in pancreatic islets or photoreceptors, eventually resulting in apoptosis in these cell types. As oligomerization precedes trans-autophosphorylation and mRNA cleavage, Ghosh *et al.* predicted that a particular threshold in IRE1 α oligomerization levels could bias this switch between survival and death. IRE1 α variants known to undergo robust activation and oligomerization underwent extra-XBP1 RNA cleavage and apoptosis, whereas cancer mutations that intermediately activate UPR signaling fail to undergo apoptosis. Application of a potent type II IRE1 α kinase inhibitor (KIRA6), which allosterically blocked RNase activity and oligomerization, prevented extra-XBP1 cleavage and apoptosis in ER-stressed cells. Finally, treatment with KIRA6 in animal models of photoreceptor or islet degeneration due to terminal UPR signaling was able to significantly preserve the functioning mass of cells. These findings suggest that the level of IRE1 α oligomerization can trigger distinct physiological outputs that dictate cell survival or death.

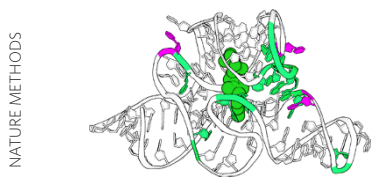
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Written by Mirella Bucci, Joshua M. Finkelstein, Catherine Goodman, Grant Miura and Terry L. Sheppard

NUCLEIC ACIDS

The SHAPE I'm in

Nat. Methods doi:10.1038/nmeth.3029



Accurately predicting the secondary structures of long RNAs from their sequences is very challenging, but generating accurate models of RNA structures can be greatly facilitated by incorporating information obtained from chemical probes. Siegfried *et al.* have modified their previously published 'SHAPE' method—in which 2'-hydroxyl groups in flexible regions of the RNA backbone are selectively acylated with a reactive chemical probe—so that it can be used to rapidly generate highly accurate models of long, structured RNAs. Whereas previous versions of SHAPE identified these modified 2'-hydroxyl groups because they inhibit reverse transcription, this new 'SHAPE-MaP' method records sites of 2'-hydroxyl-modified RNA as mutations introduced during reverse transcription. Next-generation sequencing is then used to directly identify the positions and quantify the relative frequencies of the modified nucleotides. The authors first showed that SHAPE-MaP could replicate existing knowledge about ligand-induced conformational changes in the *Escherichia coli* thiamine pyrophosphate riboswitch (see image). They then used the strategy to examine the 9,200-nucleotide HIV-1 RNA genome and

identified 15 RNA regions that were predicted to have well-defined structures, several of which were not previously known. The authors determined that two newly identified pseudoknots may have an important function *in vivo*, as the introduction of silent mutations that disrupted the secondary structures of these pseudoknots yielded viruses with reduced fitness in cell-based assays.

JMF

PROTEIN ENGINEERING

Plasmids out, codons in

ChemBioChem, doi:10.1002/cbic.201400075; ChemBioChem, doi:10.1002/cbic.201402235

The use of orthogonal tRNA-synthetase pairs for stop codon reassignment has enabled the insertion of a huge diversity of noncanonical amino acids into proteins. However, expression levels of these non-natural proteins in cells remain disappointingly low in most cases, which is thought to be a consequence of the multiple checkpoints in translational fidelity and competing release factors. Wang *et al.* now identify two ways that bacteria actively repress the non-natural system. The authors created a plasmid containing genes for the commonly used *Methanococcus jannaschii* tyrosyl tRNA and its synthetase as well as a GFP containing an internal TAG codon. After a small number of passages, nonfluorescent bacteria emerged as dominant over the fluorescent cells owing to the insertion of transposons within the synthetase gene, disabling it. When only fluorescent cells were passaged, the authors observed 16-fold increases in expression of YdII, a putative thioesterase, which the authors demonstrate plays an active part in