ER stress PERKs up an miRNA

Byrd et al. describe how a microRNA (miRNA) fine-tunes a cell’s response to ER stress.

When stressful conditions disrupt the ER’s normal function, the cell activates an unfolded protein response (UPR) that restores the organelle’s ability to fold and export proteins to the secretory pathway. The transcription factor XBP1 is a key component of the UPR that boosts ER capacity and cell survival by upregulating factors such as molecular chaperones and vesicle transport proteins. XBP1 itself is regulated by alternative UPR-mediated splicing of its mRNA, but Byrd et al. wondered whether expression of the transcription factor might also be controlled by miRNAs.

The researchers identified an miRNA called miR-30c-2* that targets the 3’-untranslated region of the XBP1 mRNA. miR-30c-2* was induced in cells subjected to ER stress. Its upregulation depended on the protein kinase PERK (another key component of the UPR), which drove miR-30c-2* production by activating the transcription factor NF-κB. Overexpressing miR-30c-2* reduced the levels of XBP1 and its target genes in stressed cells, whereas blocking miR-30c-2* activity had the opposite effect, boosting XBP1 levels and promoting cell survival.

Senior author Joseph Brewer thinks that miR-30c-2* helps prevent XBP1 from becoming overly active during the UPR. In some cases, it may be better for stressed cells to die instead of struggling to adapt to limited ER function. Brewer now wants to investigate whether NF-κB also regulates miR-30c-2* and XBP1 downstream of other signals, such as the pathways that upregulate B cells’ secretory capacity when they differentiate into plasma cells.

Simulating segregation

Gay et al. construct a mathematical model that accurately describes how fission yeast chromosomes segregate during mitosis.

Sister chromatids must attach to microtubules from opposite spindle poles so that they will segregate to different daughter cells during anaphase. Gay et al. analyzed the dynamics of mitotic chromosomes and spindle microtubules in live fission yeast and used their measurements to build a model of chromosome segregation in silico. The model was based on the assumption that, early in mitosis, spindle microtubules attach to and detach from kinetochores at random. But the simulated chromosomes segregated promptly and faithfully as long as two activities were in place to limit incorrect kinetochore–microtubule attachments.

Meet the neighbors

Roux et al. report a new way to screen for protein–protein interactions in mammalian cells.

Existing methods for probing protein interactions have their limitations. A yeast two-hybrid screen, for example, involves expressing proteins in a non-native cell type that may not fold or modify correctly. Biochemical “pull-down” approaches, on the other hand, are limited by protein solubility and can miss weak or transient interactions. Roux et al. developed a new method called proximity-dependent biotin identification, or BioID, which relies on a promiscuous mutant of the bacterial biotin ligase BirA that biotinylates nearby primary amines, such as lysine residues in neighboring proteins.

The researchers fused the mutant ligase to human lamin-A, an insoluble component of the protein meshwork that underlies the inner nuclear membrane. When the fusion protein was expressed in cells, it localized to the nuclear envelope and biotinylated nearby proteins, which could be purified on biotin-binding beads and identified by mass spectrometry. This approach detected many nuclear membrane proteins and nuclear pore complex components known to associate with lamin-A. It also identified a previously uncharacterized protein that the authors localized to the nuclear envelope and named soluble lamina-associated protein of 75 kD, or SLAP75.

BioID identifies both a protein’s binding partners and its near neighbors, says senior author Kyle Roux. In addition to using the technique with different target proteins, Roux wants to examine how disease-linked mutations in lamin-A alter the protein’s association profile.

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