RNA SPLICING

Some don't like it hot

It's well known that heat shock causes the repression of pre-messenger RNA splicing. Reporting in *Nature*, James Manley and colleagues have now identified the repressor responsible for this effect — a general splicing repressor that is known as SRp38.

SRp38 is activated by dephosphorylation and, indeed, the authors showed that SRp38 became dephosphorylated when HeLa cells were heat shocked *in vivo*. When the cells were allowed to recover, SRp38 reverted to its phosphorylated form. Heat treatment of HeLa nuclear extracts *in vitro* also resulted in the rapid dephosphorylation of SRp38 and inhibited splicing activity. When nuclear extracts were left to recover, splicing activity was restored.

Direct evidence came from depletion studies, in which the depletion of SRp38 from the nuclear extracts of heat-treated cells, or from heat-treated nuclear extracts, restored splicing activity. Subsequent addition of dephosphorylated SRp38 to the depleted nuclear extract restored splicing inhibition.

Glutathione-*S*-transferase (GST) 'pull-down' assays revealed a mechanistic clue. The U1 small nuclear ribonucleoprotein (snRNP) bound strongly to the GST–SRp38 fusion protein, but only when SRp38 was in its dephosphorylated form. Previous studies had shown that the interaction between another splicing factor, ASF/SF2, and U1 snRNP stabilizes the U1 snRNP recognition of 5′ splice sites. Manley and colleagues now show that SRp38 in its dephosphorylated form results in a strong decrease in ASF/SF2–U1-snRNP complex formation at 5′ splice sites. This suggests that dephosphorylated SRp38 represses splicing by interacting with U1 snRNP, and thereby interferes with U1 snRNP recognition of 5′ splice sites.

So, is SRp38-mediated repression of splicing important for cell survival under stress conditions? The answer is yes — even though SRp38-deficient cells are viable, they are temperature sensitive and recover more slowly from heat shock than wild-type cells. So, the authors conclude that SRp38 "…represses splicing following heat shock, at least in part, to prevent the possible accumulation of inaccurately spliced mRNA".

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPER Shin, C. *et al.* Dephosphorylated SRp38 acts as a splicing repressor in response to heat shock. *Nature* **427**, 553–558 (2004)

ORGANELLE INHERITANCE

Keep your independence

An ongoing debate about mammalian Golgi-membrane inheritance might now have been resolved by a paper from Pecot and Malhotra in *Cell*. The debate has been whether Golgi membranes remain independent during mitosis or fuse with the endoplasmic reticulum (ER). And a new assay, designed by the authors, now indicates that Golgi membranes keep their independence.

During mitosis, Golgi membranes are initially fragmented into large blobs, and these blobs are then fragmented further to produce the 'Golgi haze'. It has been hard to resolve whether the Golgi fragments in this haze remain separate from the ER during mitosis, or whether, at some point, they fuse with the ER and then re-emerge.

The new assay is based on the ability of two proteins — FK506-binding protein (FKBP) and FKBP–rapamycin-associated protein (FRAP) — to interact in the presence of the ligand rapamycin. Pecot and Malhotra made two constructs: one that that contained FKBP fused to the Golgi-protein sialyltransferase (ST–FKBP) and another that contained FRAP fused to the ER-protein human invariant-chain protein (Ii–FRAP).

If the Golgi fuses with the ER at any time during mitosis, ST–FKBP will bind to Ii–FRAP in the presence of rapamycin and be retained in the ER. However, if the Golgi retains its independence, ST–FKBP will be found in re-forming Golgi membranes in daughter cells after mitosis. Pecot and Malhotra found that, after mitosis, ST–FKBP was localized to re-forming Golgi fragments in the presence and absence of rapamycin, which indicates that the Golgi does not fuse with the ER during mitosis.

To confirm their conclusion, the authors used brefeldin A (a drug that induces ER–Golgi fusion) and rapamycin to show that ST–FKBP becomes trapped in the ER during mitosis if the ER and Golgi membranes are artificially fused. This result indicates that if Golgi proteins did cycle through the ER during mitosis, ST–FKBP would have been retained in the ER in this assay.

The authors believe that this work has resolved the debate regarding mammalian Golgi-membrane inheritance, and it now seems that "…the biogenesis of the Golgi apparatus in daughter cells is from preexisting Golgi elements rather than *de novo*".

Rachel Smallridge

References and links

ORIGINAL RESEARCH PAPER Pecot, M. Y. & Malhotra, V. Golgi membranes remain segregated from the endoplasmic reticulum during mitosis in mammalian cells. *Cell* **116**, 99–107 (2004)