Making ends meet: a role of RNA ligase RTCB in unfolded protein response

Witold Filipowicz

The unfolded protein response (UPR) monitors the protein folding capacity of the endoplasmic reticulum. One of the UPR branches includes an unusual cytoplasmic splicing reaction leading to removal of an intron from an mRNA encoding a key UPR transcription factor. The cleavage step of the process is well characterized in both yeast and animals, but the animal enzyme responsible for exon ligation has remained a mystery. Recent reports, including a paper in this issue of *The EMBO Journal*, identify RTCB as the RNA ligase operating during UPR in mammals and *C. elegans*.

See also: J Jurkin *et al* (December 2014), SG Kosmaczewski *et al* (December 2014) and Y Lu *et al* (December 2014)

The unfolded protein response (UPR) is an intracellular signaling pathway activated by endoplasmic reticulum (ER) stress that arises from the accumulation of unfolded proteins in the ER lumen (Walter & Ron, 2011; Moore & Hollien, 2012). In metazoan animals, UPR is mediated by three different ER transmembrane proteins that monitor conditions in the lumen: (i) IRE1 initiates cytoplasmic splicing of mRNA encoding a transcription factor X-box binding protein 1 (XBP1); (ii) protein kinase PERK phosphorylates translation initiation factor eIF2- α ; and (iii) ATF6, a transcription factor precursor that is activated by proteolysis. Together, these three UPR branches induce transcriptional and translational responses that increase protein folding capacity and decrease the folding load in the ER. IRE1 is the only branch operating in *Saccharomyces cerevisiae* and is consequently the most studied and molecularly best-understood UPR

pathway. The intra-lumenal sensor domain of IRE1 detects unfolded proteins and promotes lateral oligomerization of IRE1 in the ER membrane, which results in activation of the IRE1 cytoplasmic endoribonuclease domain (Fig 1). When activated, IRE1 excises an intron in *XBPI* mRNA (or its yeast counterpart *HAC1*), initiating an unusual splicing reaction distinct from the nuclear spliceosome-mediated processing. In *S. cerevisiae*, exons resulting from *XBPI*/*HAC1* cleavage are joined by RNA ligase TLR1, a multi-functional enzyme also involved in splicing of intron-containing tRNAs. *XBPI*/*HAC1* splicing allows the synthesis of functional proteins that direct expression of factors alleviating ER stress (e.g. protein chaperones) (Walter & Ron, 2011; Moore & Hollien, 2012).

Although the IRE1-mediated cleavage of *XBPI*/*HAC1* mRNA occurs by a similar mechanism in most organisms, how the resulting exons are ligated during UPR in animals has remained a puzzle. In fact, the chemistry of RNA ligation during tRNA splicing in animals is different to that in yeast (Filipowicz & Shatkin, 1983; Laski *et al*, 1983; Fig 1), and recent purification and cloning of human tRNA ligase revealed a complex of five proteins, with the catalytic subunit represented by RTCB and an additional protein, archease, acting as a stimulatory co-factor (Popow *et al*, 2011, 2014). The question of whether these proteins are also involved in *XBPI* mRNA splicing has been answered by “yes” in three recent papers that identify RTCB as the RNA ligase operating during UPR. Experimentation in the papers by Jurkin *et al* (2014) and Kosmaczewski *et al* (2014) capitalized on the prior discovery of RTCB’s role in tRNA splicing, while Lu *et al* (2014) employed a

genome-wide RNA interference (RNAi) screen using a smart synthetic biology approach.

The decision by Lu *et al* to use a non-biased screen was no doubt motivated by a report that depletion of RTCB by RNAi in HeLa cells does not impair *XBPI* mRNA splicing (Iwawaki & Tokuda, 2011). However, the occurrence of *RTCB* among the top candidate genes certainly caught their attention. Using mouse *RTCB* inducible-knockout embryonic stem cells, they found that depletion of RTCB as well as affecting tRNA splicing also impaired accumulation of the spliced (s) mRNA form and its translation product *XBPI_s*. This was accompanied by lower expression of established *XBPI_s* target genes including *XBPI* itself, as *XBPI_s* influences its own expression through a positive feedback loop. Importantly, this phenotype was rescued by re-expression of wild-type but not catalytically inactive RTCB. Demonstration that *XBPI* mRNA splicing can be reconstituted *in vitro* with recombinant RTCB and the IRE1 nuclease domain provided the final evidence for the ligase’s role in UPR.

Similarly, Jurkin *et al* (2014) first looked for evidence of RTCB activity in *XBPI* mRNA splicing in HeLa cells and extracts and found that lysates of cells with RNAi knockdown of RTCB or archease were deficient in the ligation of *XBPI* mRNA exons. However, consistent with the data of Iwawaki and Tokuda (2011), depletion of RTCB alone had almost no effect on *XBPI* splicing in intact cells. Marked repression of its maturation and the occurrence of downstream effects of *XBPI* depletion required the simultaneous knockdown of RTCB and archease. Hence, archease is a generic RTCB co-factor, active in both tRNA and *XBPI* mRNA splicing. In

Figure 1. Processing of XBP1 mRNA during UPR.

(A) Binding of unfolded proteins to the intra-lumenal domain (in orange) of IRE-1 causes its oligomerization and activation of the ribonuclease domain (in red). The IRE1 kinase domain, which is important for IRE1 regulation, is in blue. Cleavage and ligation of XBP1 mRNA is schematically shown below, with exons represented by thick and the intron by thin lines. (B) Chemistry of the RNA ligation step. The IRE1-mediated cleavage of pre-mRNA in the stem-loop border structures produces 2',3'-cyclic phosphate and 5'-OH termini in both yeast and metazoa. In yeast, two exons are ligated via a 2'-phosphomonoester, 3',5'-phosphodiester linkage, with the linking phosphate originating from the end of the downstream exon undergoing 5'-phosphorylation. The 2'-phosphate is removed from the final spliced product. In animals, RTCB catalyzes formation of a regular phosphodiester bond. The exon 3'-terminal cyclic phosphate and its fate during ligation reaction are marked in red.

its presence, even the low RTCB levels remaining after knockdown were sufficient to catalyze effective XBP1 exon ligation.

Jurkin *et al* also investigated the importance of RTCB *in vivo*, in the situation when the UPR pathway is part of a physiological developmental program. Differentiation of B cells to antibody-producing plasma cells is associated with large XBP1-dependent expansion of ER in anticipation of massive antibody secretion (Walter & Ron, 2011; Moore & Hollien, 2012). Jurkin *et al* generated a conditional mouse model in which RTCB is deleted specifically in all mature B-cell types. In response to lipopolysaccharide stimulation, RTCB-deficient B cells failed to induce XBP1_s. They also had a lower percentage of plasmoblasts with significantly affected immunoglobulin secretion due to the disorganized ER structure. Moreover, immunization of mice carrying RTCB-deficient B cells resulted in a reduction in cells secreting antibodies and in antibody titers in the serum.

Kosmaczewski *et al* (2014) investigating the role of RTCB in *Caenorhabditis elegans* found that RTCB loss-of-function worms grow to mature but sterile adults. Use of reporters dependent on IRE1/XBP1 showed that induction of ER stress with tunicamycin

failed to activate the reporters in mutant worms. ER stress in the mutants did not induce XBP1_s mRNA accumulation, but this defect was rescued by expression of wild-type but not inactive RTCB.

Like the authors of the other two papers, Kosmaczewski *et al* were concerned with distinguishing the roles of RTCB in UPR and in tRNA splicing: Depletion of tRNAs could impact both translation and growth. In *C. elegans*, all genomic copies of tRNA^{Leu(CAA)} and tRNA^{Tyr(GUA)} contain introns. By constructing transgenic animals expressing intron-less copies of these tRNA genes, the authors reversed the effects of RTCB deletion on worm lifespan and growth under unstressed conditions. Unexpectedly, RTCB^{-/-} worms expressing intron-less tRNAs still had highly penetrant phenotypes independent of UPR: They were defective in vulval development and were sterile due to the failure of germ cells to mature to oocytes. The authors speculate that these UPR- and tRNA splicing-independent phenotypes are due to additional substrates of RTCB ligase or to tRNA fragments accumulating in RTCB-deficient worms, for example, tRNA halves originating from intron-containing tRNA genes present in the genome. tRNA-derived fragments were found recently to have strong deleterious

effects on various aspects of cell activity (Anderson & Ivanov, 2014). A further possibility is that artificially created intron-less tRNA^{Leu(CAA)} and tRNA^{Tyr(GUA)} are not fully functional due to lack of certain nucleoside modifications. Some modifications are introduced into tRNAs at the level of intron-containing precursors (Grosjean *et al*, 1997), and deficiencies in modification could affect tRNA performance, possibly in a cell- or mRNA-specific fashion (Gu *et al*, 2014).

XBP1 mRNA splicing is unusual with respect to not only the hairpin-like exon/intron borders (Fig 1) and the proteins involved but also its cytoplasmic localization. Previous work demonstrated the ER association of HAC1/XBP1 mRNA and thus its availability for rapid processing upon activation of IRE1. All three reports verify the presence of RTCB, and also archease, in the cytoplasm with considerable fractions associated with ER, all consistent with a role in XBP1 splicing. Lu *et al* also demonstrated that RTCB and IRE1 interact with each other even in the absence of UPR induction.

The physical association of IRE1 with RTCB raises interesting possibilities. In animals, IRE1 not only acts in the processing of XBP1 mRNA but also in a process known

as RIDD (regulated IRE1-dependent decay), mediates degradation of ER-associated mRNAs encoding membrane and secretory proteins, which relieves the folding burden of the ER (Walter & Ron, 2011; Moore & Hollien, 2012). Recently, Cho *et al* (2013) found that the RIDD-derived mRNA fragments produced in response to intra-luminal activation of IRE1 by cholera or Shiga toxins activate the RIG-I innate immunity pathway, stimulating expression of NF- κ B and inflammatory cytokines. Similar effect, depending on phosphorylated 3'-ends, was previously described for RNA fragments generated by the IRE1-related ribonuclease RNase L (Malathi *et al*, 2007). Irrespective of whether induced by toxins or regular UPR, the RIDD-generated mRNA fragments bear 5'-OH and 2',3'-cyclic phosphate termini, which makes them ideal substrates for RTCB. Since RTCB has a very relaxed specificity and can efficiently ligate any RNA with the proper termini (Filipowicz *et al*, 1983; Popow *et al*, 2011), it is possible that it also functions to mitigate innate immune signaling by catalyzing intramolecular ligation (circularization) of RIDD RNA fragments.

In summary, the three studies provide convincing evidence that RTCB not only processes intron-containing tRNAs in animals but also splices XBP1, a key regulator of UPR that is also important for priming specific cells for high secretory activity, such as plasma cells. As always, such studies also raise many new questions. Is RTCB indeed involved in the ligation of substrates other than tRNAs and XBP1 mRNA, and is it possibly involved in repair/religation of RNAs incidentally cleaved by cellular endoribonucleases? Do other components of the RTCB

complex also play a role in XBP1 (and tRNA) splicing? If so, what are their specific functions? It would be nice if answering these questions takes less than the decade or so consumed in the search for the mysterious RNA ligase carrying XBP1 splicing.

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