Review of: "Live imaging of the co-translational recruitment of XBP1 mRNA to the ER and its processing by diffuse, non-polarized IRE1α"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

The article “Live Imaging of the co-translational recruitment of XBP1 mRNA to the ER and its processing by diffuse, non-polarized IRE1a” by Gomez-Puerta et al. represents a commendable account of analysis of the recruitment of XBP1 mRNA in Hela Cell using single molecule imaging approach. The article addresses and tests several fundamental and important issues concerning the activation of Unfolded Protein Response (UPR) in mammalian cell using the Hela cell as a model. UPR is a vital intracellular signalling pathway that regulates the ability of the protein folding of endoplasmic reticulum (ER) especially during the massive protein unfolding within the ER lumen. Dysfunction of this pathway is involved in a number of disease states including neurodegenerative disorders, apoptosis and cancer. Understanding the molecular mechanisms of this pathway is therefore seminal to develop therapeutic interventions into these diseases.
XBP1 (X-box binding protein) mRNA encodes a vital transcription factor that plays a pivotal role in the activation of this pathway. During ER stress induced by several drug, eg. tunicamycin or thapsigargin the XBP1 mRNA undergoes a non-canonical splicing by IRE1a protein that is present in ER surface. Unspliced XBP1 mRNA is recruited to the ER surface for IRE1α-mediated splicing during ER stress and this recruitment is supposed to be translation dependent.
In this work, the authors used single molecule imaging approach to scrutinize the mechanism of recruitment of individual XBP1 mRNA into ER surface, which they have shown requires a specific motif present in unspliced XBP1 protein that is dubbed HR2 motif. Furthermore, they showed that IRE1α-mediated splicing triggers the release of the XBP1 mRNA from the ER membrane to cytosol in response to ER stress. Intriguingly, they noted that XBP1 transcripts are not recruited into pre-formed large IRE1α clusters, which assemble only upon the overexpression of fluorescently-tagged IRE1α during ER stress. They authors pointed out that the IRE1α assemblies are homogeneously distributed on the ER surface.
This investigation involves the appropriate use of diverse microscopic and live-cell imaging technologies that allows the authors image and monitor the movement, recruitment and attachment of the individual XBP1 transcript to the ER surface. Using the XBP1-MS2 reporter-fluorescent MS2 coat protein pair they image the individual XBP1 transcripts (both WT and various mutant versions) on the ER surface as diffraction-limited spots and compared their ER association with the previously benchmarked Gaussia transcripts, which tightly attach to the ER surface. They also checked the translation dependence of the XBP1 recruitment, requirement and consequence of splicing on the attachment and release of this transcript to and from ER surface using a modified single molecule imaging technique.

The work represents a fine piece of investigation, which unfolds many unknown facts about the recruitment and mobilization of XBP1 mRNA and in my opinion is worth publication in a top ranking journal. It employed a combination of

molecular biological and single-molecule imaging approach that nicely addressed several outstanding and important questions in this area and many of which were resolved from the finding of this work.

However, I have several comments/concerns, which the authors should address and extend further experiments to clarify these concerns. They are listed below.

Comments

1. Size of the illustrations describing various constructs are too small and not legible. A bigger version of these figures should be presented.
2. Fig. 1E. I suggest that the author should include similar frames as shown in Fig. 1D for the HR2 mutant for a better and clearer comparison.
3. Fig. 1F: The co-localization of red dots (WT HAC1) and grey dots (Gaussia) are significantly high in the fourth quadrant which indicates that a large fraction of WT HAC1 mRNAs are strongly associated with ER surface, whereas the the correlation of yellow and grey dots are poor suggesting negligible amount of HR2 mutant is associated with ER.
4. However, in Fig. 1F, substantial overlap of red dots (WT) and yellow dots (HR2 mutant) were observed especially in the junction of first and second quadrant above the line indicating an instantaneous diffusion co-efficient of 0.06 mm^2/sec.
5. Fig. 3D: It appears that the ER stress was induced for at least 3 hours before the imaging session as mentioned in the text. It should be mentioned in the legend. Moreover, a representative image under the control condition should be included in this panel. Otherwise, it is difficult to analyse and interpret the relevance of the changes of the value of ER association
6. Fig. 3C: For WT reporter XBP1 transcript, it was observed that treatment of the Hela cells with 5mg/ml TM for 3hrs led to the decreased ER association of the spliced transcript, which was reversed when the cells were additionally treated with 4m8C, an inhibitor of the IRE1a endo-ribonuclease activity abolishing the splicing of XBP1 mRNA. The authors interpret this data as: after 3 hrs of induction of ER-stress, the spliced WT XBP1 transcripts are released from the ER sites as free transcripts in the cytosol, which is supported by the finding that inhibition of splicing inhibits this release. Although this interpretation is perfectly acceptable, a kinetic datasets showing the time-dependent ER association of the WT XBP1 transcript at different times under control condition (no TM), after addition of TM till 4 hours and in presence of 4m8C would have been helpful to validate this interpretation.