Title:"Studies on the role of Rab-GTPase Yptlp in the nuclear mRNA surveillance in Saccharomyces cerevisiae" [Index No.: 66/17/Life Sc./25]

ABSTRACT

Nuclear degradation of pre-*HAC1* mRNA and subsequent targeting of the resulting precursor mRNA plays a vital role in the activation as well as attenuation of Unfolded Protein Response (UPR) in *Saccharomyces cerevisiae*. Unfolded Protein Response (UPR) is an intracellular signalling pathway that responds to stress causing a burden of unfolded proteins (ER stress) in the ER lumen. To mitigate this situation, baker's yeast *Saccharomyces cerevisiae* produce a huge burst of Hac1p, a transcription factor that activates genes encoding ER chaperones. Hac1p is encoded by *HAC1* pre-mRNA harboring an intron and a bipartite element (BE) at its 3′-UTR, which undergoes a reversible and dynamic intra-nuclear 3′→5′ mRNA decay by the nuclear exosome/CTEXT at various phases of UPR. Accelerated decay, in absence of stress leads to a pre-*HAC1* mRNA pool, the majority of which lacking the BE, thereby undergoing inefficient targeting to Ire1p foci consequent to diminished splicing and lower production of mature Hac1p. ER-stress leads to its diminished decay, producing an increased abundance of pre-*HAC1* mRNA population carrying an intact BE leading to its accelerated recruitment to Ire1p foci followed by increased splicing and increased production of mature Hac1p.

Rab-GTPaseYpt1p, which was previously implicated in the ER to Golgi shuttling of proteins was also demonstrated to be involved in the regulation of UPR signalling dynamics by promoting the decay of *HAC1 m*RNA. This finding suggests a potential involvement of Ypt1p in the regulatory mechanism for selection and recognition of the *HAC1* pre-mRNA by the nuclear exosome/CTEXT. In this study we show that physical association (as detected by RNA-immunoprecipitation) of Rab-GTPase Ypt1p with *HAC1*-pre mRNA facilitates the recruitment (as detected by ChIP) of NNS/CTEXT/nuclear exosome on to the pre-*HAC1* mRNA and stimulates its nuclear decay in absence of stress. In presence of stress, reduced amount of Ypt1p becomes associated with the pre-*HAC1* thereby lowering the recruitment of exosome/CTEXT/NNS (as detected by Co-immunoprecipitation) on to it, which leads to the production of huge amounts of pre-*HAC1* mRNA with an intact BE. Regulation by Ypt1p relies on its characteristic nuclear localization in absence of ER-stress causing its strong association with pre-*HAC1* mRNA at its 3′-UTR that promotes a sequential recruitment of Nrd1-Nab3p-Sen1p (NNS) complex → CTEXT → nuclear exosome onto the pre-*HAC1* mRNA that eventually stimulates its selective nuclear decay. During induction of UPR, Ypt1p rapidly relocalizes to the cytoplasm with its consequent dissociation from pre-*HAC1* mRNA thereby causing a decreased recruitment of NNS/CTEXT/Nuclear exosome with its consequent diminished 3′→5′ degradation by the exosome. This reversible mechanism ensures a timely activation of UPR and its prompt attenuation following the accomplishment of homeostasis. These findings will help to gain an insight into the mechanism of intra-cellular signalling pathway UPR that is accomplished by differential degradation at the post-transcriptional level.

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