

The Subcellular Localisation of EhRRP6 and Its Involvement in Pre-Ribosomal RNA Processing in Growth-Stressed *Entamoeba histolytica*

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Abstract : The eukaryotic exosome complex plays a pivotal role in RNA biogenesis, maturation, surveillance and differential expression of various RNAs in response to varying environmental signals. The exosome is composed of evolutionary conserved nine core subunits and the associated exonucleases Rrp6 and Rrp44. Rrp6p is crucial for the processing of rRNAs, other non-coding RNAs, regulation of polyA tail length and termination of transcription. Rrp6p, a 3'-5' exonuclease is required for degradation of 5'-external transcribed spacer (ETS) released from the rRNA precursors during the early steps of pre-rRNA processing. In the parasitic protist *Entamoeba histolytica* in response to growth stress, there occurs the accumulation of unprocessed pre-rRNA and 5' ETS sub fragment. To understand the processes leading to this accumulation, we looked for Rrp6 and the exosome subunits in *E. histolytica*, by in silico approaches. Of the nine core exosomal subunits, seven had high percentage of sequence similarity with the yeast and human. The EhRrp6 homolog contained exoribonuclease and HRDC domains like yeast but its N- terminus lacked the PMC2NT domain. EhRrp6 complemented the temperature sensitive phenotype of yeast *rrp6Δ* cells suggesting conservation of biological activity. We showed 3'-5' exoribonuclease activity of EhRrp6p with in vitro-synthesized appropriate RNAs substrates. Like the yeast enzyme, EhRrp6p degraded unstructured RNA, but could degrade the stem-loops slowly. Furthermore, immunolocalization revealed that EhRrp6 was nuclear-localized in normal cells but was diminished from nucleus during serum starvation, which could explain the accumulation of 5'ETS during stress. Our study shows functional conservation of EhRrp6p in *E. histolytica*, an early-branching eukaryote, and will help to understand the evolution of exosomal components and their regulatory function.

Keywords : *entamoeba histolytica*, exosome complex, rRNA processing, Rrp6

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