Mechanistic insights into the mRNA poly(A) tail machinery

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Almost every eukaryotic mRNA has a 3' poly(A) tail that contributes to post-transcriptional regulation of gene expression by regulating translation and mRNA stability. The poly(A) tail is added to pre-mRNAs in the nucleus by the 1 MDa cleavage and polyadenylation factor (CPF). CPF is thought to contain 15 different protein subunits, most of them essential for viability in yeast. CPF cleaves the mRNA with an endonuclease subunit (Ysh1), adds a poly(A) tail with its polymerase (Pap1), and regulates transcription via two protein phosphatases (Ssu72 and Glc7). How these enzymes are coordinated and assembled remains poorly understood. We are using a hybrid approach combining structural (cryo-EM, x-ray crystallography, NMR), biochemical, biophysical and genetic techniques to gain insights into the molecular mechanisms of the poly(A) machinery. Using native mass spectrometry, we show that the CPF complex is assembled via three modules based around its enzymatic activities. Further, we will present new structural and biochemical insights into the polyadenylation module.