Structure determination of a mRNA polyadenylation complex by cryo-EM

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Protein-coding genes in eukaryotes are transcribed by RNA polymerase II (Pol II) as precursor messenger RNAs (pre-mRNAs). These pre-mRNAs must undergo 5′ capping, splicing and 3′-end processing before they can be transported to the cytoplasm for their translation into proteins. 3′-end processing involves over 20 different protein factors that also co-ordinate transcription termination. The cleavage and polyadenylation factor (CPF) is an essential component of the 3′-end machinery that cleaves pre-mRNA transcripts and adds the 3’ polyA tails. PolyA tails confer stability to the mRNA and are important for efficient translation into proteins. Despite the fundamental importance of CPF, there is little structural information available and we are still far from understanding its molecular mechanisms. Here, I describe how we determined the cryo-EM structure of a core scaffold complex of CPF to 3.6 Å resolution. We were able to build atomic models de novo into the map by using improved tools in COOT for cryo-EM. Our structure comprises three proteins that are essential for polyadenylation and act as a hub to assemble other CPF subunits and accessory factors on RNA.