Structural basis for NTP specificity of non-canonical CutA nucleotidyltransferase

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During the course of its lifecycle, eukaryotic mRNA undergoes several types of modifications at different stages of its maturation. These modifications significantly affect the mRNA fate. One such modification is a post-transcritional addition of untemplated nucleotides to mRNA 3'-end. Apart from well-known polyadenylation, the presence of homo- or heteropolymeric stretches containing nucleotides other than adenosine has been reported for protein-coding transcripts in multiple organisms, including fungal, plant and animal species.
CutA is a non-canonical nucleotidyltransferase identified in filamentous fungi *Aspergillus nidulans,* which belongs to DNA polymerase β superfamily. *In vivo*, it adds short CUCU-rich extensions to 3'-ends of mRNA, which in turn leads to deadenylation-independent decapping and eventually mRNA degradation. Our previous biochemical studies carried out using recombinant CutA from *Thielavia terrestris* demonstrated that although the enzyme is able to processively polymerize only adenosines, it indeed displays an unusually high specificity towards cytidines as compared to other known terminal nucleotidyltransferases, and it synthesizes predominantly tails terminating with two cytosines.
Our present work involves determination of crystal structures of *T. terrestris* CutA catalytic domain alone and in complex with substrate and product. The apo structure of CutA was solved by Se single-wavelength anomalous diffraction. Based on the structural analysis further biochemical characterization was performed on selected mutants to get deeper insight into catalytic properties of the enzyme: 1) high specificity towards CTP; 2) exclusive processivity towards ATP; and 3) a complete lack of activity for GTP as an incoming nucleotide and RNA substrate ending with guanines.