Biochemical Analysis of the Nuclease Module of the Human Ccr4-Not Deadenylase Complex

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Abstract

In eukaryotic cells, the shortening and removal of the poly(A) tail (deadenylation) of cytoplasmic mRNA is a key step in mRNA degradation. The Ccr4-Not complex is well-characterised as a major deadenylase enzyme involved in mRNA deadenylation. The complex contains two catalytic subunits: Ccr4 and Caf1. Currently, it is unclear whether the Ccr4 and Caf1 catalytic subunits work cooperatively, or whether the nuclease components have unique roles in deadenylation. To facilitate the biochemical analysis of deadenylase enzymes, we have developed a fluorescence-based deadenylase assay, which is sensitive, quantitative and suitable for micro-well plate formats. We demonstrate the utility of the new assay for the discovery of small molecule inhibitors of the human Caf1/CNOT7 deadenylase enzyme. These compounds may become useful tools to investigate the contribution of the Caf1/CNOT7 in deadenylation. Furthermore, to understand the requirement and relative contributions of the ccr4 and Caf1 catalytic subunits, we therefore developed a method to express and purify a minimal human BTG2•Caf1•Ccr4 nuclease sub-complex from bacterial cells. By using chemical inhibition and well-characterised inactivating amino acid substitutions, we demonstrate that the enzyme activities of Caf1 and Ccr4 are both required for deadenylation. We propose a mechanism, in which the Caf1

and Ccr4 subunits cooperatively participate in mRNA deadenylation by the Ccr4-Not complex.