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Title: IDENTIFICATION AND STRUCTURAL BASIS OF A NOVEL CELLULAR INHIBITOR OF THE INTEGRATOR CLEAVAGE MODULE

Abstract

The Integrator complex was initially found to be required for 3' end processing of non-coding small nuclear RNAs through its association with RNA polymerase II (RNAPII). However, the metazoan-specific 15-subunit complex has been shown to function as a broad transcriptional regulator. Integrator represses gene expression through an RNA endonuclease to cleave nascent RNA and associated PP2A to remove phosphorylation important for elongation. Despite its apparent importance to transcription, we have yet to discover the full scope of how Integrator activity is regulated, thus representing a knowledge gap in the field. The Integrator RNA endonuclease activity is found within Integrator subunit 11 (IntS11). We have shown that IntS11 heterodimerizes with IntS9 and has additional interactions with IntS4 to form the Integrator cleavage module (ICM). We conducted extensive purifications of individual *Drosophila* Integrator subunits to identify a previously uncharacterized *Drosophila* protein, dBrat1, as a specific cofactor unique to dIntS11. Surprisingly, most dIntS11 is associated with dBrat1 in the cytoplasm, and dIntS11 accumulation is highly dependent on dBrat1 expression. Using Cryo-EM, we solved the structure of dBrat1 in complex with dIntS11 at 3 Angstroms resolution. The dIntS11-dBrat1 structure shows that the conserved C-terminus of dBrat1 extends into the active site of dIntS11, mimicking the RNA substrate. Additionally, domains within dBrat1 prevent dIntS11 interaction with dIntS4, preventing formation of the ICM. Taken together, these observations indicated that dBrat1 is an inhibitor of dIntS11. Our current work will elucidate the functional implications of the INTS11-BRAT1 complex in human systems. BRAT1 re-localizes to the nucleus upon DNA damage and is an important scaffolding component of the DNA damage repair response. We have developed BRAT1 null and Auxin inducible INTS11 degron lines to assess the function of the complex. These and fluorescent localization studies will allow us to assess the INTS11-BRAT1 function upon DNA damage.