Genetics Day Poster Submission

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Title: IDENTIFICATION AND STRUCTURAL BASIS OF A POTENTIAL LICENSING FACTOR 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 17-subunit complex has been shown to function as a broad transcriptional regulator. Integrator represses gene expression through RNA endonuclease activity to cleave nascent RNA and associated PP2A to remove phosphorylation critical 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). Here, we conducted extensive purifications of individual Integrator subunits in Drosophila and human cell lines to identify a previously uncharacterized protein, dBrat1, as a specific cofactor that binds dIntS11 and dIntS9. Using cryo-EM, we solved the structure of dBrat1 in complex with dIntS11 at 3 Å resolution. The dIntS11-Brat1 structure shows that the conserved C-terminus of dBrat1 extends into the active site of dIntS11, mimicking the RNA substrate. Further, purification of human BRAT1 or INTS11 reveals that these two proteins likely associate with INTS9 and WDR73 in the cytoplasm. Using this information, we have now solved a new structure containing INTS9, INTS11, and BRAT1 and we are currently working towards solving structures that also contain WDR73. Functionally, depletion of BRAT1 in HCT116 or hES cells leads to a profound loss of Integrator function causing significant misprocessing of UsnRNA, indicating a critical requirement for Brat1 association. Altogether, these results suggest that cytoplasmic INTS11-containing complexes may function to promote nuclear Integrator function through a still undefined mechanism that may involve localization and assembly of INTS9/11 into the complete Integrator complex.