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Title: IDENTIFICATION OF THE Z3 MODULE AS A MODULE OF THE INTEGRATOR COMPLEX

Abstract: The Integrator complex has been classically defined as a broad transcriptional regulator important for regulation of both coding and noncoding RNAs. The modularity of the Integrator complex has been more recently defined through structural studies and has led to the discovery of new roles for the complex as a whole. The modules of Integrator actively function with RNA Polymerase II (RNAPII) to serve specific roles that are critical for transcriptional regulation, including endonuclease activity and phosphatase activity. Not currently understood, is how Integrator is recruited to the promoter-proximal region of genes in order to facilitate termination, thus representing a knowledge gap in the field. The Z3 complex was initially discovered to associate with Integrator through Immunoprecipitation/Mass-Spectrometry (IP/MS) studying the Integrator complex and its involvement in transcription regulation. This Z3 complex consists of three zinc fingers: ZMYND8, ZNF592, and ZNF687. Little is understood about the molecular mechanisms of the Z3 complex, but rare mutations in the zinc fingers have caused several neurological disorders or cancers, similar phenotypes to mutations in specific Integrator subunits. To begin to characterize the importance of the Z3 complex to Integrator, we created individual 293T cells stably expressing doxycycline-inducible FLAG-tagged Z3 proteins and used these lines to generate nuclear extracts. From those extracts, we performed purification of each Z3 protein using anti-FLAG affinity resin and conducted mass spectrometry to identify associated factors. These approaches revealed that each Z3 subunit not only pulls down the other two subunits confirming the presence of a likely trimeric complex, but also each subunit of the Integrator complex and multiple members of the Pre-Elongation Complex. Functional studies are currently underway using an Auxin Inducible Degron (AID) system in order to rapidly deplete each zinc finger individually. We propose a model in which the Z3 complex is actually a new module of the Integrator complex that is involved in modulating the function of Integrator at promoter-proximal regions of nascent RNA transcripts.