Analyzing Mutations of Spt7 Protein That Disrupt Interaction with SF3B Subunits

Arryn T. Harris, Peyton Spreacker, Rachel Stegeman and Dr. Vikki Weake
Department of Biochemistry, University of Purdue
Edwin C. Acosta
Department of Biology, University of Puerto Rico

ABSTRACT

Proper transcription, the process of converting DNA to RNA, is crucial for the health and viability of an organism. This process is regulated by many proteins, such as co-transcriptional activators; one being the protein complex known as Spt-Ada-Gcn5-acetyltransferase, or SAGA. While much is known about the roles of SAGA in cell processes, how SAGA’s subunits promote functionality is still unknown. The focus of this study is to analyze the purpose of SAGA’s SF3B subunits. These subunits are also found in the spliceosome, the compound responsible for generating mature RNA. SAGA has no known functions relating to this process, so the reason the SF3B components are in SAGA is unclear. Spt7, another SAGA subunit, interacts with both SF3B subunits. In this study, a yeast two hybrid assay was performed where different Spt7 mutants were screened. This was done by transforming yeast with Spt7 mutants, analyzing the protein interactions and sequencing the mutants to determine their mutations. A key result of this study is in the determining that the two SF3B subunits interact with different regions of Spt7. Although the overall goal is to find an Spt7 mutant that does not interact with the SF3B components but still maintains interaction with other SAGA subunits, we now have a better idea of what type of Spt7 mutant is needed. This discovery will lay the foundation for future experiments where a mutated SAGA with no SF3B components will be expressed in Drosophila melanogaster and analyzed to determine the function of SF3B subunits in SAGA.

KEYWORDS

Transcription, SAGA, SF3B3, SF3B5, Spt7, protein interaction