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Determining the binding between SAGA subunits and spliceosomal components

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ABSTRACT

Proper gene regulation is vital to the health and development of an organism. Determining the relationship between splicing, transcription, and chromatin structure is vital for understanding gene regulation as a whole. There have been previous studies linking these elements pairwise; however, no evidence exists for a direct link between all three. Recent data shows that splicing components of the U2 small nuclear ribonucleic protein (snRNP) co-purify with Spt-Ada-Gcn5-acetyltransferase (SAGA), a highly conserved transcriptional co-activator and chromatin modifier. We hypothesize that SAGA binds with splicing components through a multi-protein binding surface with certain core components based on preliminary yeast two-hybrid data. Here, we examine the specific binding partners between SAGA and splicing components utilizing the yeast two-hybrid system in *spt7Δ Saccharomyces cerevisiae* as a validation for the preliminary yeast two-hybrid performed, producing recombinant proteins through sequence and ligation-independent cloning (SLIC) and Baculovirus transfections to obtain purified proteins, and co-immunoprecipitation (co-IP) to detect specific protein-protein interactions from recombinant proteins. Yeast two-hybrid results reveal that Spt7 is necessary for the transcription of reporter genes used in this assay. Therefore, this assay cannot validate previous results or detect false positives. Currently, recombinant proteins are being produced to perform co-IPs to test direct protein interactions. The results from these experiments will demonstrate the type of binding between SAGA subunits and splicing factors and provide direct evidence of a link between all three of the elements of gene regulation.

KEYWORDS

SAGA, U2 snRNP

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