Identifying the Role of a protein degradation factor (Elc1) in regulating mRNA processing factors.

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Abstract

The processing of mRNA is a critical for protein synthesis. Our experiments show that Elc1, a protein degradation factor found in yeast, could be involved in regulating mRNA processing following certain types of DNA damage.

Our hypothesis is that Elc1 degrades Cleavage and Polyadenylation factors (CPF) a key protein complex involved in processing mRNA. Data from previous experiments indicate that DNA damage causes reduced levels of the CPF complex, but not when using a strain that lacks Elc1.

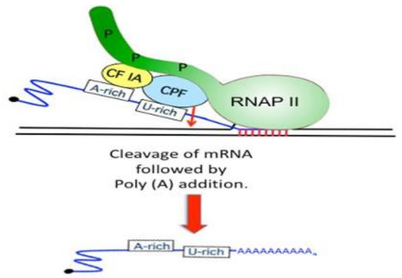
Currently we are attempting to purify the CPF complex using a specific yeast strain that contains a tagged version of one of the CPF components. We will be using antibody conjugated beads to isolate the CPF from yeast extracts. The goal is to test our hypothesis and check if purified Elc1 can in fact degrade this purified CPF in-vitro.

Methods and Material

In order to test our hypothesis, CPF must be purified from yeast culture. Elc1 has already been purified in prior experiments by Monica Mack.

Through consultation of tandem affinity purification (TAP) tags, we determined the proper yeast strain with the Protein A and Calmodulin Binding Peptide (CBP) tags attached to a protein of the Cleavage and Polyadenylation (CPF) complex. We then streaked YPD plates in order to get individual colonies of the proper yeast strain. Since a large amount of protein is needed to conduct our future experiment, we used four 50 mL cultures to inoculate four larger 1000 mL cultures. We are currently working on purifying the CPF complex.

Introduction



The processing of mRNA is a necessary component of creating proteins. The transcribed mRNA is cleaved and a tail structure is added. Cleavage and Polyadenylation factors (CPF) are involved in the cleavage and polyadenylation steps of mRNA processing.

When RNA polymerase is stalled due to DNA damage, Elc1(a protein degradation factor found in yeast) degrades the largest subunit of RNAPII.
Our data suggest that Elc1, is involved in regulating mRNA processing and also that CPF is degraded in response to certain types of DNA damage that stalls RNAPII.

Our hypothesis is that Elc1 is involved in the degradation of CPF.

References

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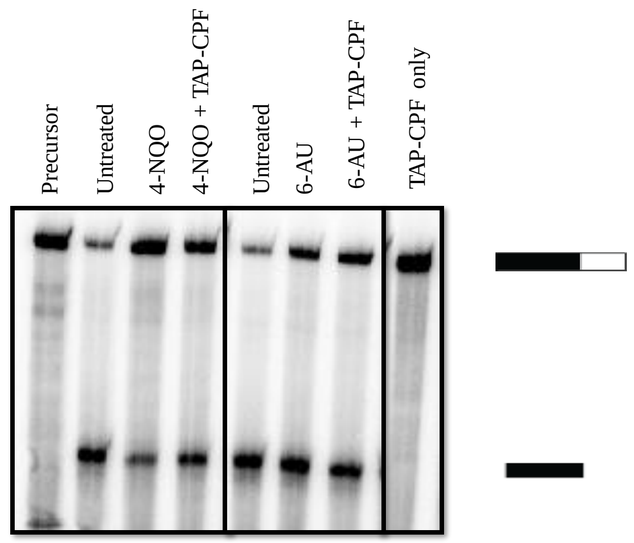
Results



TAP-CPF partially rescues cleavage activity in 4-NQO treated extracts

To check if reduced levels of CPF play a critical role in the inhibition of cleavage, we added back TAP purified CPF to 4-NQO treated extracts and performed cleavage assays using a radiolabelled RNA substrate. Adding back TAP-CPF rescued cleavage activity in 4-NQO treated (DNA damaged) extracts (lane 4). However, TAP CPF did not rescue cleavage in 6-AU treated (non DNA damaged) extracts

Future Directions

- Protein extraction and tandem affinity purification will be carried out using IgG and calmodulin conjugated beads targeting Protein A as well as Calmodulin Binding Peptide (CBP) respectively. A Western blot will be used to check the purity of the CPF complex.
- Varying concentrations of purified Elc1 and CPF will be mixed together and analyzed through Western Blots to check if increasing amounts of Elc1 will lead to the degradation of key CPF components.