Effects of Transcription Blocking Drug on mRNA Processing Factors

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Abstract

Transcription is a process that creates messenger RNA (mRNA) from a DNA template. Following transcription, mRNA processing is required to create mature mRNA that can be translated to protein.

UV induced DNA damage slows down transcription by stalling RNAPII, the enzyme that produces mRNA. UV damage also results in reduced levels of mRNA processing and mRNA processing factor proteins. We are studying if the observed effect on mRNA processing is due to DNA damage or RNAPII stalling.

To test if the reduced level of mRNA processing factors observed after UV-induced DNA damage is due to DNA damage or simply RNAPII stalling, we treated yeast cells with 6-Azauracil, (6-AU) which stalls RNAPII in the absence of DNA damage, and analyzed mRNA processing factor levels using western blotting. Our results did not show reduced levels of mRNA processing factors following 6-AU treatment, suggesting that the observed inhibition of mRNA processing and lower levels of processing factors are a result of DNA damage and not simply RNAPII staling.

Background

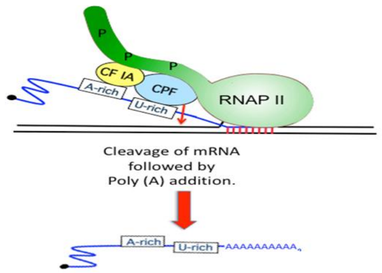

Figure 1. RNAPII. mRNA maturation involves capping the 5' end, splicing of introns and processing the 3' end of the newly transcribed mRNA. 3' end processing involves cleaving the nascent mRNA and adding a stretch of nucleotides referred to as the poly (A) tail.

RNA polymerase II, is the enzyme responsible for producing mRNA. Rpb1 is the largest subunit protein of RNA polymerase II. When DNA is damaged through UV, RNAPII cannot move along on DNA transcribing and producing mRNA as usual. The DNA damage response is activated, Rpb1 is degraded and the tail addition step of mRNA processing does not occur when this happens.

However, whether this step is blocked due to the DNA damage response or the physical blockage of RNAPII, as well as the protein factors responsible for this process are largely unknown.

Our goal is to determine which pathways and proteins are responsible for blocking mRNA processing in yeast. Our hypothesis is that certain proteins responsible for Rpb1 degradation like Rpb9, Def1 and Elc1 are involved in blocking mRNA processing.

Results

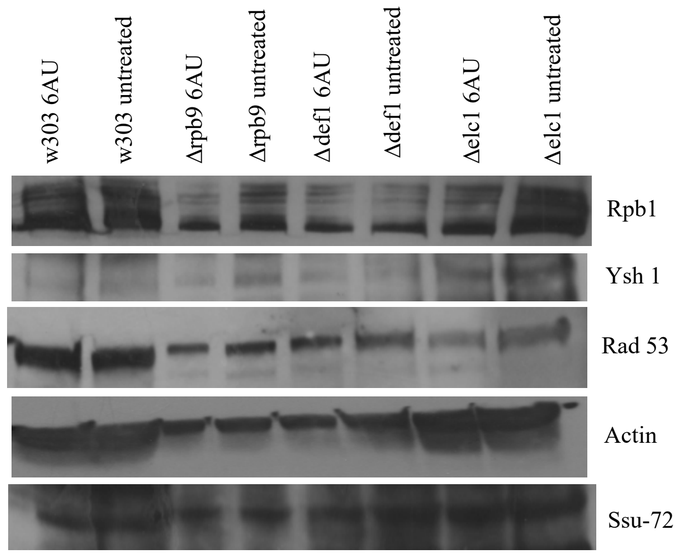

Figure 1: Western blot analyzing protein levels in WT and mutant cell extracts show now changes in protein levels following 6-AU treatment.

When WT W303, Δdef1, and Δelc1 cells were treated with 6-AU or left untreated there was no significant difference in the protein levels probed for. 6-AU is a drug that can block RNAPII in the absence of DNA damage.

Rpb1 is the largest subunit of RNAPII and is degraded following DNA damage. No change is observed in Rpb1 levels in the 6-AU treated cells.

Rad53 is a protein which is phosphorylated in response to DNA damage and shows a mobility shift on a SDS-PAGE gel. We do not observe this shift in the treated samples.

Ysh1 and Ssu-72 are mRNA processing factors and required for the formation of the 3' end of transcribed RNA. Following DNA damage, reduced levels of these processing factors are observed. However no change in levels were observed after 6-AU treatment.

Actin was used as a loading control.

Conclusions

- None of the cell lines tested showed Rpb1 degradation or a shift in Rad53 on the gel following 6-AU treatment, suggesting that 6-AU did not induce the DNA response.
- mRNA factor levels were unchanged in all the cell lines tested, either treated with 6-AU or untreated, suggesting that RNAPII stalling alone, in the absence of DNA damage, does not result in degradation of mRNA processing factors.

Materials and Methods

Bacterial Transformation

All cells were transformed with the pRS316 plasmid that allows cells to grow on media lacking uracil. This procedure used various heating and cooling stages to allow the plasmid to enter the cell.

Growing Cells on –URA Media and treatment with 6-AU

All strains were grown in media lacking uracil overnight, so that 6-AU (an inhibitor of transcription) can have an effect. Cells were treated with 6-AU at a concentration of 0.5µg/ml and harvested 1hr after treatment.

Cell extract Preparation.

Cell extracts were prepared using a Lithium acetate/NaOH extraction procedure. After overnight growth, cells were centrifuged and treated with Lithium acetate and NaOH. Cell debri was removed and proteins extracted using SDS Laemlli buffer .

Western Blot Analysis

This consisted of running gels, via electrophoresis and transferring them to a membrane. Membranes were then treated with primary and HRP conjugated secondary antibodies, followed by chemiluminescence generating substrates to quantify protein levels.

Future directions

- Analyze for other processing factor levels in these extracts to complete our analysis on mRNA processing factor levels following 6-Au treatment

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