A DNA damage independent role for a DNA damage response factor

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Abstract

mRNA processing is an essential step in mRNA maturation. mRNA processing involves intron splicing, 5' capping, and 3'end processing. 3' end processing involves 2 steps - cleavage of mRNA at a specific point and polyadenylation (the addition of adenosine nucleotides) at the end of the RNA. 3' end processing is carried out by a large multi subunit protein 3' end processing complex. Rad53 is a protein involved in the DNA damage response. It is known to amplify the DNA damage signal. The function of Rad53 independent of DNA damage has not been studied well. Our hypothesis is that Rad53 regulates 3' end processing independent of DNA damage. We show that cell extracts lacking Rad53 behave differently than wild type (WT) cells that carry the Rad53 protein. WT cell extracts are able to cleave and polyadenylate RNA in our in-vitro assay but extracts from cells lacking Rad53 are defective in cleavage, regardless of DNA damage. We used western blotting to detect specific protein levels of the 3' end processing complex in cell lines with or without Rad53. However, we don't see any significant changes in processing factor levels in WT or cells lacking Rad53, suggesting that Rad53 does not play a role in regulating 3' end processing factor levels. The next step is to check if Rad53 physically interacts with the 3' end processing complex. We will be using an immunoprecipitation experiment to test this. These studies will help determine how Rad53 regulates mRNA 3' end processing independent of DNA damage.

Background

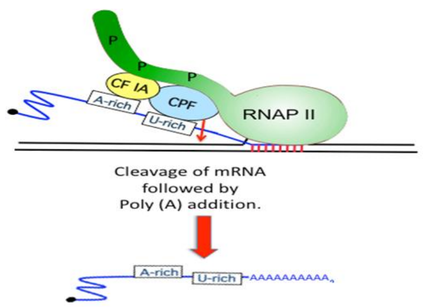

Figure 1. mRNA maturation involves capping the 5' end, splicing of introns and processing the 3' end of the newly transcribed mRNA. 3' end processing involves cleaving the nascent mRNA and adding a stretch of nucleotides referred to as the poly (A) tail.

The mRNA 3' end processing complex processes cuts a newly transcribed RNA transcripts at a specific point and adds a string of adenosine nucleotides to make mature RNA. Only then can the RNA moves into the cytoplasm to make protein.

Rad53 is a protein that is involved with DNA damage response. It is activated by phosphorylation after DNA damage and then it can phosphorylate other proteins which stop cell division and helps the cell to repair DNA damage.

Our experiments show that Rad53 plays an unexpected role in mRNA 3'-end processing.

Results and Conclusions

Extracts from cells lacking Rad53 cannot process mRNA to make mature RNA

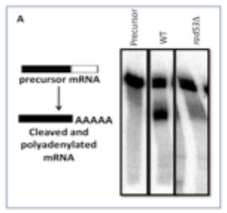

Figure 2 – Rad53 affects the cleavage step of 3' end processing in vitro. Extracts from cells lacking the DNA damage response factor Rad53 are defective in cleavage. Coupled cleavage and poly(A) addition was assayed using radioactive precursor containing the GAL7 poly(A) site and flanking sequences. Addition of WT extracts to substrate RNA results in cleaved and polyadenylated product. When extracts from rad53Δ are added, severely reduced amounts of cleaved and polyadenylated RNA is observed. Cleaved, unadenylated RNA does not accumulate, indicating that it is the cleavage step that is blocked.

Cells lacking Rad53 do not have lower levels of RNA processing factor subunits

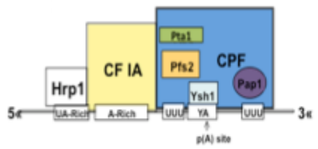

Figure 3 - The RNA processing complex is made up of several subunits. The pre-mRNA is cleaved by the endonuclease Ysh1 and the poly(A) polymerase Pap1 synthesizes the poly (A) tail. Other proteins act as a scaffold and / or bind to specific RNA sequences to facilitate cleavage at the correct site.

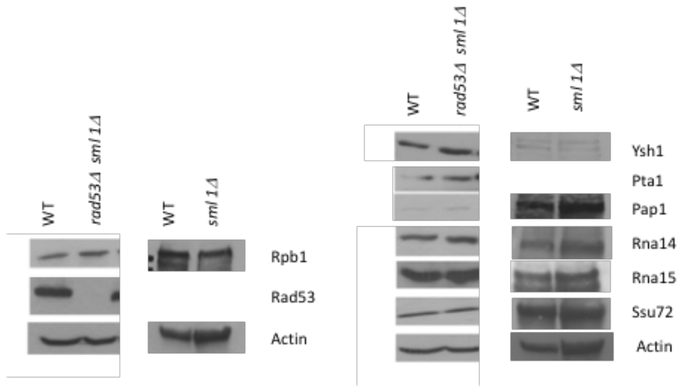

Figure 4 – mRNA processing factor subunit levels do not show any changes in levels in extracts lacking Rad53. Extracts from cells lacking Rad53 and Sml1 (a ribonuclotide reductase inhibitor) or cells lacking Sml1 only were analyzed by Western blot. The SML1 gene needs to deleted to ensure survival of cells lacking Rad53. Extracts lacking Sml1 were also therefore analyzed to check if any effect observed were specific to Rad53. A slight decrease in Rpb1 (the largest subunit of RNA polymerase) levels in observed in extracts lacking Sml1 only. However cells lacking both Rad53 and Sml1 do not show changes in Rpb1 levels. This suggests that Rad53 might be playing a role in reducing Rpb1 levels in cells undergoing transcriptional stress such as those lacking the ribonucleotide reductase inhibitor Sml1.

Materials and Methods

In vitro RNA processing assays

Radioactive RNA was prepared in-vitro using a plasmid containing the GAL7 sequence under a T3 promoter. The radioactive RNA was then added to yeast cell extracts as indicated. Processed and unprocessed RNA was then analyzed using a urea polyacrylamide gel and imaged using a phosphoimager.

Western Blotting

Proteins were extracted using LiAc and NaOH and equivalent amounts run on a 10% SDS/PAGE gel. Following transfer to a PVDF membrane the proteins were analyzed using the indicated antibodies.

Future directions

Immunoprecipitation assay to analyze for protein-protein interactions between Rad53 and RNA processing factors.

We will be using an immunoprecipitation assay where I will break open cells and mix the cell extract with beads which have an antibody against a tag on the RNA processing factor subunit PtaI.

Once we have all the proteins bound to the beads we will separate them and analyze if Rad53 actually binds to PtaI or any other the protein on the processing complex using western blotting.

References

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