Investigating the Role of Introns in Transcription-Associated Mutagenesis in Budding Yeast

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Background

The process of transcription of a gene can cause mutation of that gene\(^1\).\(^2\). This phenomenon is referred to as transcription-associated mutagenesis (TAM). TAM can occur via several processes, including the formation of R-loops (RNA:DNA hybrid plus the single-stranded nontranscribed strand), which can stall the DNA replication/repair and transcription machinery and leave single-stranded DNA vulnerable to mutagens. Introns enhance transcriptional output of genes. They are also believed to prevent R-loop-mediated TAM via co-transcriptional splicing\(^1\). However, the impact of splicing on TAM has not been thoroughly established.

Nontranscribed strand vulnerable to mutagens

Methods/Results

Yeast Transformation

Figure 1

Introns Have Been Proposed to Prevent R-loop Formation Through Co-transcriptional Splicing

Without an intron present within a gene, nascent pre-mRNA during transcription can loop back on and anneal with the transcribed DNA strand, leaving the nontranscribed strand a single-stranded state vulnerable to DNA damage. This multidimensional transcription structure makes up a R-loop. Major parts of the figure are labeled in each panel. In the “intron-containing” panel, the grey spheres/ovals are the splicing factors that have recognized and bound to splicing signals, to facilitate co-transcriptional splicing formation. The rapid co-transcriptional formation of the spliceosome because of the presence of an intron in the pre-mRNA transcript is proposed to preclude the formation of R-loops.

Adapted from Bonnet et al. (2017)

Objective

The role which intron length and location within a highly-transcribed gene may play in TAM has not been previously investigated. Here, we assessed the effect that introns of two different lengths placed either close or far relative to an inducible promoter have on the TAM rate in a budding yeast URA3 reporter gene.

Methods/Results

Yeast Transformation

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Summary of \(p\text{URA3}\) and \(p\text{GAL1}\) URA3 Mutation Rates

Yeast link and transcriptional status

The results of the fluctuation assay were used to determine mutation rates.

Conclusions & Future Directions

- Early data from this study suggested that mutation rate is highest in long-intron \(p\text{URA3}\) strain (~17 fold greater) (See Fig. 2).
- This trend was similar for the long-intron \(p\text{GAL1}\) strain (importantly, only when induced) (Figure 6)
- Interestingly, the proximal long-intron strain did not exhibit elevated TAM, and the distal long-intron strain exhibited the highest TAM rate among our strains (Figure 6).
- The spot assays shown in Figures 7-9 demonstrated that only the long-intron strains exhibited smaller colonies on uracil-deficient media, except for the proximal-long strain.
- Sequence \(URA3\) of 5-FOA-resistant clones to determine mutation spectra.
- Reverse-transcription-qPCR of each strain to assess \(URA3\) expression levels.

Future Directions

- R-loops may be reduced in short-intron strains and proximal-long strain because of efficient spliceosome formation, but are elevated in all other long-intron strains.
- Assess R-loop formation in future using DNA-RNA immunoprecipitation (DRIP) coupled with reverse-transcription-qPCR.
- TAM may play role in human genome, which contains very long introns in cancer-linked genes (e.g. \(TP53\))

References


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