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

Cedric Lansangan & Dr. Jane Kim, Ph.D.

California State University San Marcos - Department of Biological Sciences

Background

The process of transcription of a gene can cause mutation of that gene[1-3]. 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] (Fig. 1). However, the impact of splicing on TAM has not been thoroughly established.

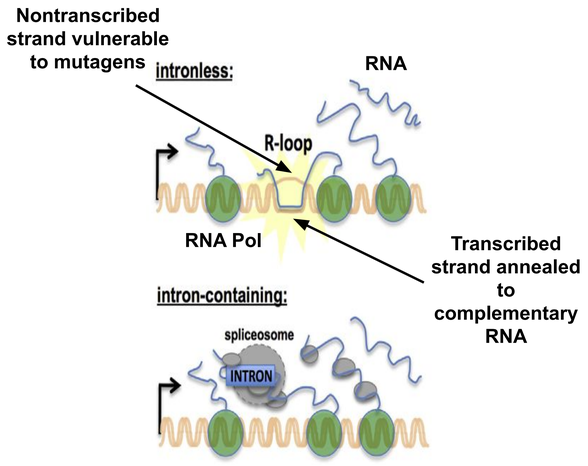

Figure 1

Introns Have Been Proposed to Prevent R-loop Formation Through Co-transcriptional Spliceosome Formation in Budding Yeast

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 in a single-stranded state vulnerable to DNA damage. This stabilized three-stranded structure makes up an 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 spliceosome 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.

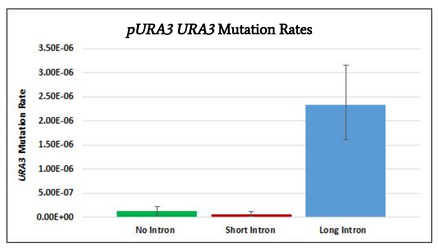

Figure 2

Constitutively-Active *URA3* Mutation Rates Were Highest in Long-Intron Strain

Error bars represent 95% confidence intervals

URA3 mutation rate of long-intron strain is ≈17-fold greater than that for short and no-intron strains ; *pURA3* = *URA3* transcription driven by endogenous promoter

Methods/Results

Yeast Transformation

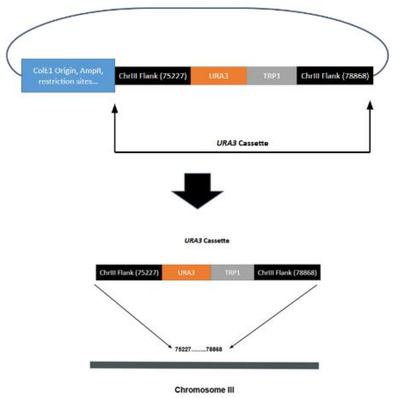

Figure 3

Illustration of Cassette Extraction and Integration

Restriction digestion of each bacterial plasmid is performed to extract and purify the *URA3* cassette. This fragment is then transformed into budding yeast, where (via the flanking homology it contains to the numbered region indicated within Chromosome 3) it is integrated through homologous recombination to replace the endogenous sequence at this locus. This specific region is replicated early and efficiently during S phase of the cell cycle through the initiation of replication from the upstream Autonomously-Replicating Sequence (ARS) 306.

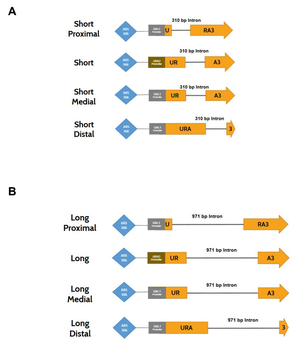

Figure 4

Illustrations of final *URA3* reporter genes integrated within the yeast chromosome 3 region as shown in Figure 3

No-Intron URA3 not shown. Blue diamond indicates upstream *ARS306* sequence. Gray rectangles indicate endogenous *URA3* promoter. Brown rectangles indicate inducible *GAL1* promoter. *URA3* exon lengths represent approximate location of intron (medial = middle of gene, proximal = close to promoter, distal = far from promoter). A) Short-Intron reporters ; B) Long-Intron reporters

Fluctuation Assay

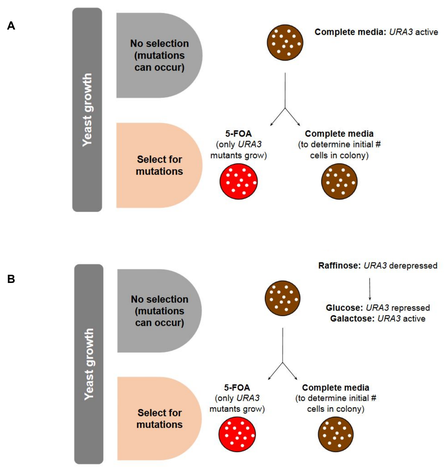

Figure 5

Fluctuation Assay Schemes Were Used to Assess Mutation Rates of *pURA3* and *pGAL1* Reporter Genes

Fluctuation assays consist of a nonselective growth phase to allow mutations to occur, followed by a selective growth phase to determine the number of mutations per cell per generation (mutation rate). A) Scheme for *pURA3* strains ; B) Scheme for *pGAL1* strains

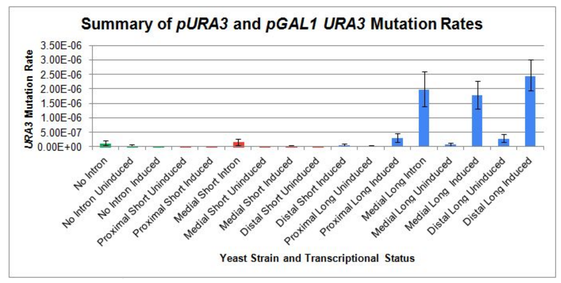

Figure 6

The Distal-Long Strain Exhibited the Highest Mutation Rate in this Study

Error bars represent 95% confidence intervals calculated for the trial most representative of all of the trials performed for each strain. We used the most-representative trial data and 95% confidence intervals instead of averaging among the trials because the two versions of our data were not different.

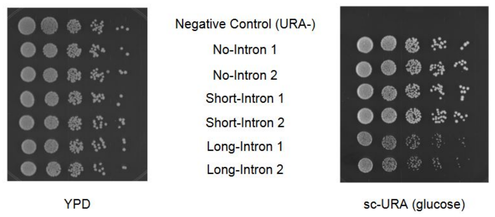

Figure 7

The Long-Intron *pURA3* Strain Exhibited Slow Growth on Uracil-deficient Media

5 microliter "spots" of 5-fold dilutions pipetted on synthetic-complete media lacking uracil and 3 microliter "spots" of the same dilutions on complete media (YPD).

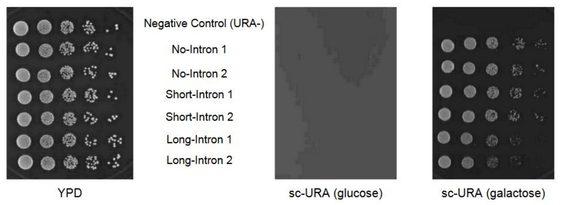

Figure 8

The Long-Intron *pGAL1* Strain Exhibited Similar Slow Growth on Uracil-deficient Media

5 microliter "spots" of 5-fold dilutions pipetted on synthetic-complete media lacking uracil with either glucose or galactose and 3 microliter "spots" of the same dilutions on complete media (YPD).

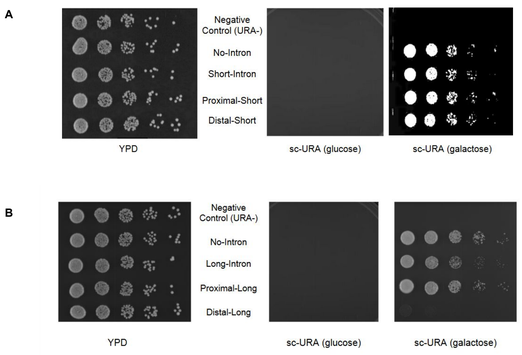

Figure 9

The Distal-Long Strain Exhibited Extremely-Slow Growth on Uracil-deficient Media

5 microliter "spots" of 5-fold dilutions pipetted on synthetic-complete media lacking uracil with either glucose or galactose and 3 microliter "spots" of the same dilutions on complete media (YPD). A) Short Distal and Proximal ; B) Long Distal and Proximal.

Conclusions & Future Directions

- Early data from this study suggested that mutation rate is highest in long-intron *pURA3* strain (~17 fold greater) (See Fig. 2).
- This trend was similar for the long-intron *pGAL1* 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.
- 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*[4])

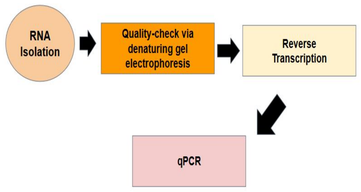

Figure 10

***URA3* RT-qPCR Study Scheme Can Quantitatively-Evaluate Either *URA3* mRNA Transcripts or *URA3* DNA:RNA Hybrid Pulldown Samples**

After isolating RNA from appropriately-grown yeast strains, or isolating the RNA species of the DNA:RNA hybrid, we will use a technique called denaturing gel electrophoresis in order to quality-check the RNA obtained. We will then reverse-transcribe the RNA to cDNA, which will finally be used to perform quantitative PCR (qPCR)

Acknowledgements

Thank you to the entire Kim lab (Dr. Jane Kim, Meghan O'Donnell, David Papp, Patricia Diangkinay, Karla Medina, Terrance Haanen, Sophia Hernandez, Ariel Duran, Berenice Arguello, Clarissa Garcia, and Gabi Ramirez), my thesis committee (Dr.s Jane Kim, James Jancovich, and Betsy Read), and everyone I have met and interacted with at CSUSM.

All of you helped to get me where I am today, and I appreciate you all.

Research in Dr. Kim's laboratory is supported by an NIH SCORE Grant (SC3GM127198)

References

1. Bonnet, A., Grosso, A.R., Elkaoutari, A., Coleno, E., Presle, A., Sridhara, S.C., Janbon, G., Géli, V., de Almeida, S.F. and Palancade, B., 2017. Introns protect eukaryotic genomes from transcription-associated genetic instability. *Molecular cell*, *67*(4), pp.608-621.
2. Brooks, Michael D., Monika L. Burness, and Max S. Wicha, 2015. Therapeutic implications of cellular heterogeneity and plasticity in breast cancer. *Cell stem cell* 17.3: 260-271.
3. Juneau, K., Miranda, M., Hillenmeyer, M.E., Nislow, C. and Davis, R.W., 2006. Introns regulate RNA and protein abundance in yeast. *Genetics*.
4. https://p53.fr/tp53-information/tp53-knowledge-center/26-knowledge-center/4-the-tp53-gene