How Cell Stress and 3' End Alterations Control the Metabolism of a Cellular Non-Coding RNA

Thomas Rivas, Jennifer Kugel, and James Goodrich

Department of Chemistry and Biochemistry, University of Colorado Boulder, Boulder, CO

University of Colorado Boulder

Abstract

RNA Polymerase II (Pol II) canonically acts as a DNA-dependent RNA polymerase (DdRP), using double stranded DNA to synthesize protein-encoding mRNAs and some non-coding RNAs. Pol II also has RNA-dependent RNA polymerase activity (RdRP), which uses an RNA as a template to synthesize RNA. An example of Pol II RdRP activity is the 3' end extension of the non-coding B2 RNA to generate extended B2 (eB2) RNA. B2 RNA binds to Pol II and is a substrate for a Pol II-dependent 18 nucleotide RdRP-catalyzed extension to form eB2 RNA. eB2 RNA can be detected in cells and has a reduced half-life as compared to B2 RNA.

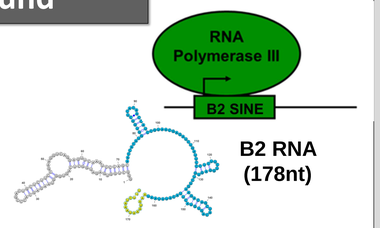
B2 RNA is transcribed by Pol III from Short Interspersed Elements (SINEs), which exist in over 350,000 copies in the mouse genome due to retrotransposition. Upon cellular stress, transcription of non-coding B2 RNAs from B2 SINEs is greatly increased, thereby increasing the likelihood of retrotransposition of B2 SINEs. The RdRP activity of Pol II could control the levels of B2 RNA post-transcriptionally by generating eB2 RNA to promote its degradation, thereby impacting the frequency of retrotransposition.

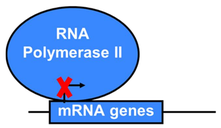
To study the effects of 3' end extension and cellular stress on the lifecycle and metabolism of B2 RNA we are developing a heterologous expression system. Because the B2 SINE sequence is embedded in many mouse mRNA transcripts (e.g. UTRs and introns), it is impossible to perform hybridization and PCR-based experiments to distinguish between a Pol III-derived B2 RNA and those sequences within mRNA transcripts. To overcome this, we express B2 and eB2 RNA in human cells, which do not contain B2 SINEs, using a human Pol III promoter. The expressed RNAs are the correct size, have the correct 5' ends, have the correct 3' secondary structure, and exogenous eB2 has decreased stability compared to B2 RNA. Therefore, the heterologous system mimics characteristics of B2 RNA and eB2 RNA in mouse cells.

Using this system, ongoing experiments are investigating how extension alters the cellular localization of B2 RNA and retrotransposition efficiency in unstressed and stressed cells. Altogether, these experiments will determine the interplay between B2 RNA expression, Pol II RdRP extension, cellular localization, and cellular stress on the life cycle of this ncRNA.

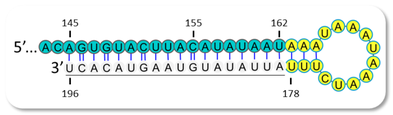
Background

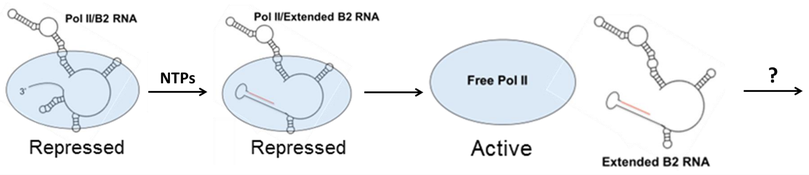
- B2 SINEs comprise ~5% of the mouse genome.
- B2 RNA is massively upregulated upon cell stress through transcription by RNA Polymerase III.





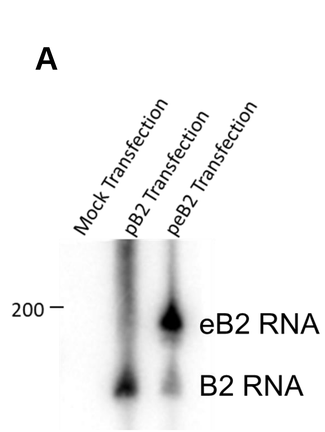
- B2 RNA binds to the active site cleft of RNA Polymerase II to prevent Pol II from binding to promoter DNA, thus repressing transcription.[1,2]
- Pol II extends B2 RNA in a template specific manner to form eB2 RNA in cells.[3]
- Extension destabilizes B2 RNA/Pol II complexes.

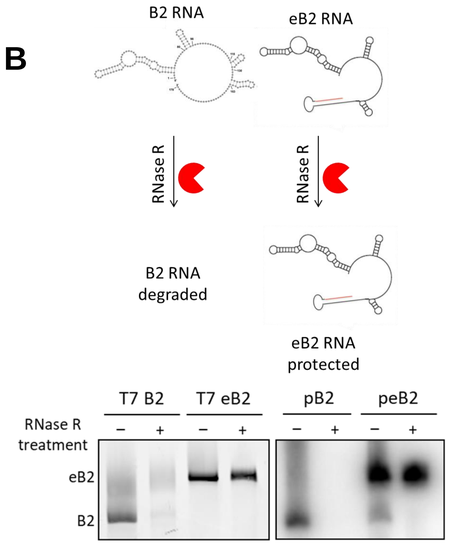




Heterologous Expression of B2 RNA

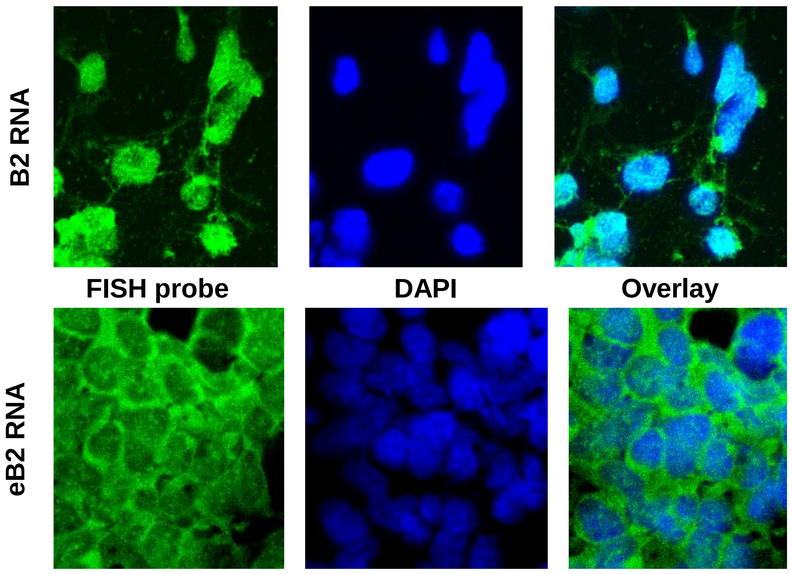
Human HEK 293 cells are transfected with a plasmid expressing either B2 RNA (pB2) or eB2 RNA (peB2) under the control of the human Pol III U6 promoter. Expressed RNAs are the correct size by northern blot (A) and are folded correctly as determined by RNase R degradation (B).



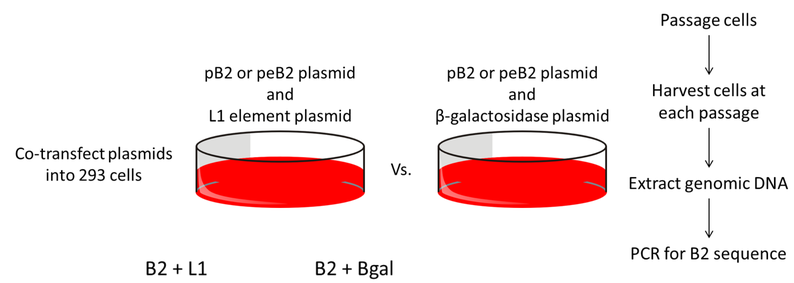


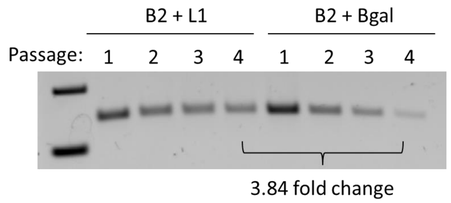
Extension alters the localization of B2 RNA

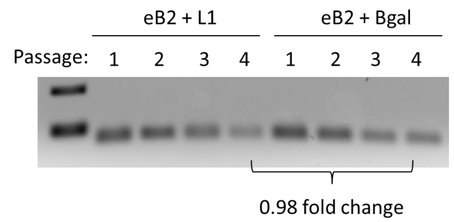
Using this heterologous expression system allows us to use RNA FISH (fluorescence *in situ* hybridization) to determine the localization of B2 and eB2 RNAs in cells. At resting conditions, B2 RNA is nuclear while eB2 RNA is cytoplasmic.



Extension blocks retrotransposition of B2 RNA







Preliminary experiments show that B2 RNA can retrotranspose into the genome with an almost 4-fold enrichment over background (e.g. cells without the L1 plasmid). Conversely, eB2 RNA does not retrotranspose with the same efficiency, as there is no enrichment over background at any passage number.

Future Directions

1. How does cell stress affect the localization and retrotransposition of B2 and eB2 RNAs?
2. Can we identify potential processing pathways by immunofluorescence experiments?
3. What are the biochemical differences between B2 and eB2 RNA that prevent retrotransposition?

Funding

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The Lab

References

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