Guide RNA

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BMS 265
5/7/09
What is Insertion/Deletion RNA Editing?

- The addition or deletion of U residues from a pre mRNA transcript.

- Unedited mRNA: A G

- Edited mRNA: A U G

- Unique to the mitochondrial genome of Kinetoplastid Trypanosomes

- Usually necessary for a functional open reading frame
Major Transcript of the Frameshifted coxl Gene from Trypanosome Mitochondria Contains Four Nucleotides That Are Not Encoded in the DNA

A Model for RNA Editing in Kinetoplastid Mitochondria: “Guide” RNA Molecules Transcribed from Maxicircle DNA Provide the Edited Information
What is a gRNA?

- 50-70 nt RNA species containing a 5’ triphosphate and 3’ poly U tail

- “Characteristic” double hairpin motif.
  (Schmid et. Al. 1995)

- Largely encoded by the minicircles of the Kinetoplastid mitochondrial genome.
gRNA Biogenesis
transcription from minicircles

Grams et al. 2001

gRNA polycistron

3’ transcribed gRNAs are degraded
gRNA Biogenesis
Addition of a U Tail

PPP  gRNA  KRET1
gRNA
Mechanism of Action

1. gRNA/pre-mRNA binding

2. pre-mRNA cleavage

3a. U insertion via TUTase action

3b. U deletion via ExoUase action

4. Re-ligation of mRNA fragments
1. gRNA/pre-mRNA binding

Poly U tail

3' UUUU

| *** |

5' ACCAGGGA

MRP1/2

Anchor

CUGGUCN_{10} 5' gRNA

| | | | |

GACCGG 3' Pre-mRNA
2. Pre-mRNA Cleavage

Poly U tail acts to tether the cleaved fragments of the pre-mRNA together.

20S Editosome

Endonuclease?

Cleavage site at first unpaired nucleotide of the pre-mRNA target.
3a. U insertion

TUTase will add Uridylates to the 3’ end of the 5’ fragment

\[\text{3’} \text{UUUUUU} \quad \text{AAGGA} \quad \text{U–UAUAUACUGGGUC} \quad \text{N}_{10} \quad \text{5’} \text{gRNA}\]
\[\text{5’} \text{ACCAGGGAA} \quad \text{AUAUAUGACCGG} \quad \text{3’Pre-mRNA}\]

U
3a. U insertion

U addition/deletion results in pairing of gRNA template to mRNA through Watson-Crick and G:U base pairing.
3b. U Deletion

20S Editosome

5′ ACCAGGGAAU

3′ UUUUUU

| *** |

CUGGUCN_{10}  5′ gRNA

5′ gRNA

3′ Pre-mRNA

ExoUase removes unpaired U
4. Ligation

3' UUUUUUUAAGGA-U-UAUAUACUGGGUCN_{10} 5' gRNA

5' ACCAGGGAAUUUUUUAUUAUAUGACCAG 3' Pre-mRNA
4. Ligation

3’ UUUU....UAUGACCAG 5’
gRNA

5’ ACCAGGGAGUACUGGUUCN10 5’
Pre-mRNA

20S Editosome
Ligase
1. gRNA/pre-mRNA binding

Editing proceeds 3’ to 5’ along the pre-mRNA
<table>
<thead>
<tr>
<th>New Nomenclature</th>
<th>Alternate Names</th>
<th>Function</th>
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<tbody>
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<td>mHel61p</td>
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<td>KRET1</td>
<td>RET1/ 3’ TUTase</td>
<td>Adds polyU tail to gRNA</td>
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<td>MRP1</td>
<td>TbgBP21/ Ltp28/ CfgBP29</td>
<td>Assists gRNA/premRNA binding</td>
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<td>TbgBP25/ Ltp26/ CfgBP27</td>
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<tr>
<td>RBP16</td>
<td>RBP16</td>
<td>binds gRNA and regulates gRNA utilization</td>
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Stuart et al. TRENDS. 2005
gRNA other stuff

Example members
http://rna.bmb.uga.edu/kiss/
http://dna.kdna.ucla.edu/trypanosome/database.html

Role in human Disease
gRNA’s are non pathogenic but they are critical to the survival of the Trypanosome.

Potential as a Tool
Maybe, if you study Trypanosomes
Thanks!