Definitions of Terms Used in Uridine Insertion/Deletion RNA Editing:

Cryptogene: A gene whose transcript is edited.

Guide RNA or gRNA: A short 3'-uridylated RNA that can form a perfect duplex (except for the oligo[U] tail) with a stretch of mature edited mRNA. G-U base pairing is allowed.

Anchor duplex: the RNA duplex formed by hybridization of the 5' end of the gRNA and the mRNA sequence just downstream of the first editing site in an editing block.

Pre-edited region or sequence: Sequence that will be edited in the mature RNA.

Unedited region or sequence: Sequence that is never edited.

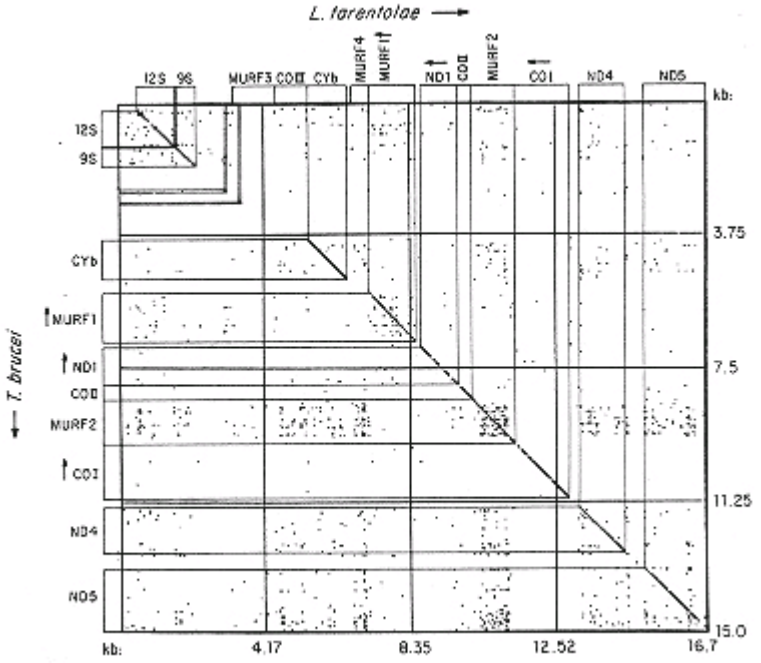
Editing block: Edited mRNA sequence mediated by a single gRNA.

Editing domain: Edited mRNA sequence mediated by 2 or more overlapping gRNAs.

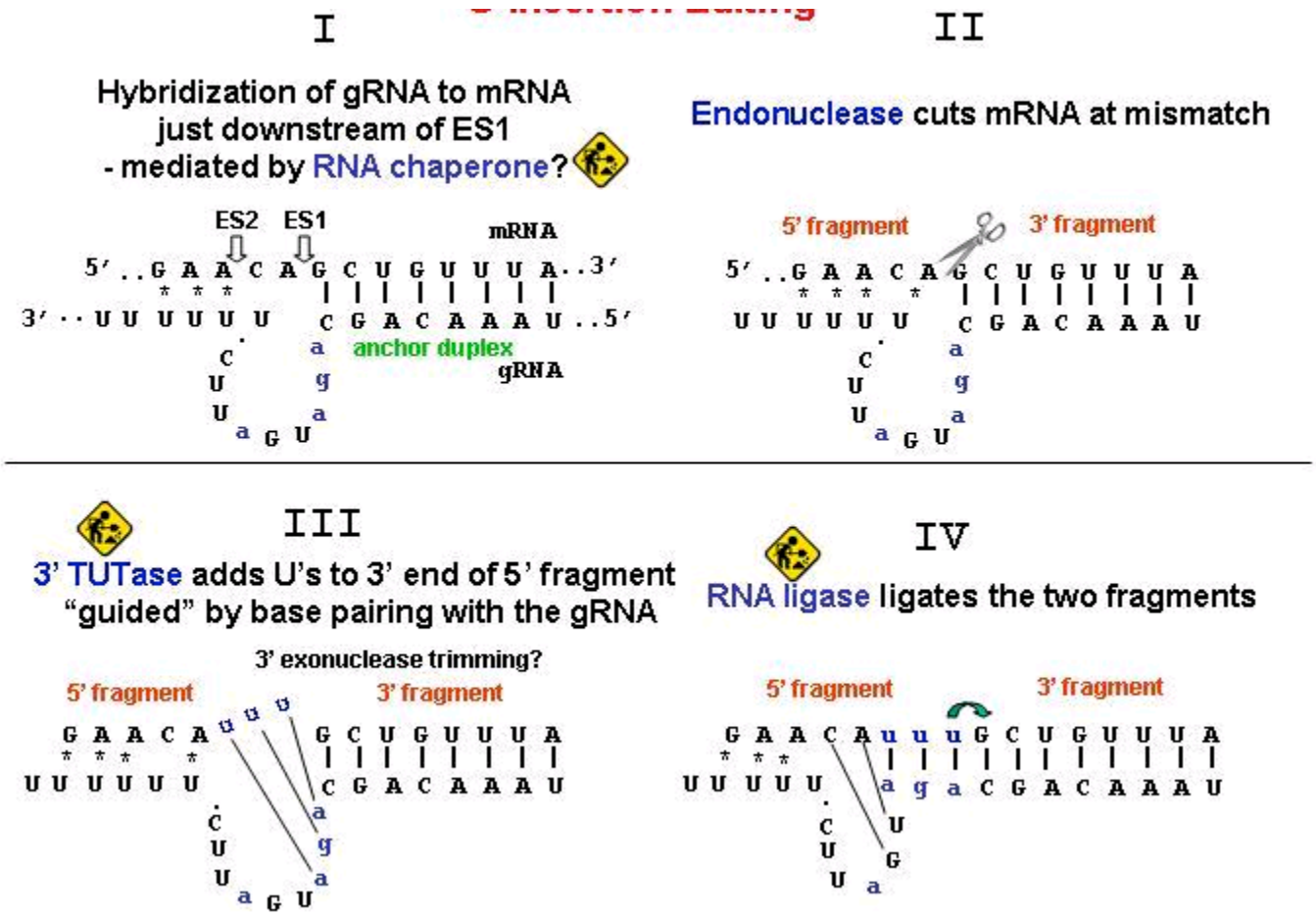
Pan-edited gene: extensively edited.

3' oligo[U] tail: The string of non-encoded U's at the 3' end of the gRNA.

gRNA-mRNA chimeric molecule: A molecule which consists of a gRNA 5' which is covalently linked at the 3' end to an mRNA, usually at an editing site.


L. tarentolae
12S
9S
MURF3
COIII
CYb
MURF4
MURF1
ND1
COII
MURF2
COI
ND4
ND5
kb:
T. brucei
3.75
7.5
11.25
15.0
4.17
8.35
12.52
16.7

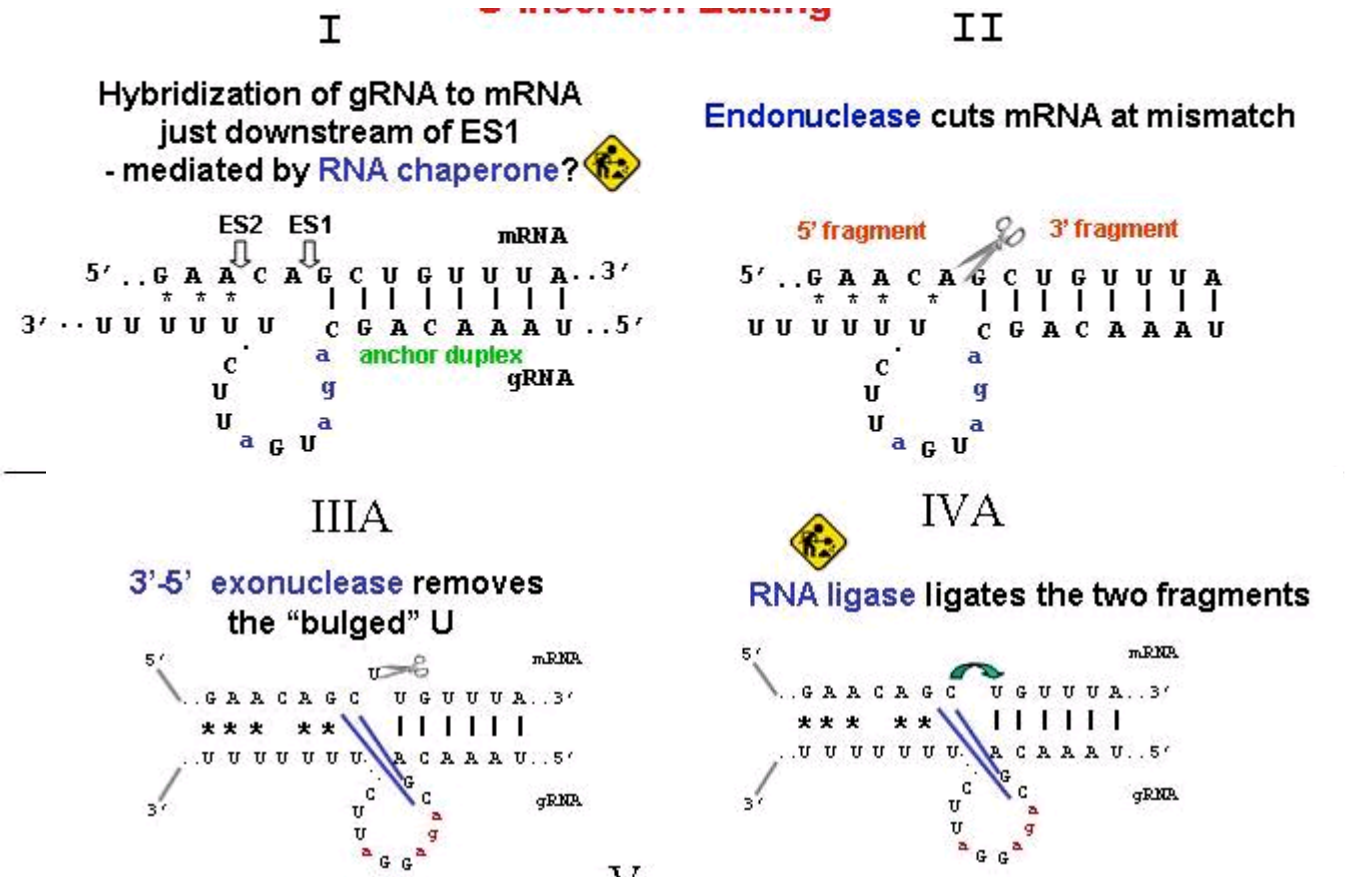
Model for U-Insertion RNA Editing



Activites needed for U-insertion:

Endonuclease – to cut the mRNA
3'-TUTase – (terminal uridyl transferase) to add Us to 3' end of 5' fragment
RNA ligase – to ligate the cut mRNA back together

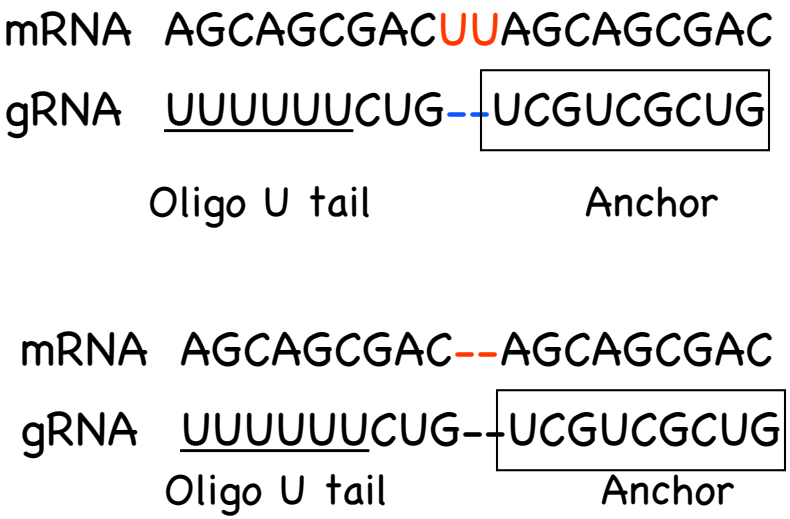
Model for U-Deletion RNA Editing



Activites needed for U-deletion editing:

Endonuclease – to cut the mRNA
3'-5' exonuclease – to remove the unpaired Us
RNA ligase – to ligate the cut mRNA back together

Deletion editing



If the U's in the mRNA aren't base paired in the gRNA, they are removed

Simple, right?

Insertion editing

mRNA AGCAGCGAC--AGCAGCGAC

gRNA UUUUUUCUGAAUCGUCGCUG

Oligo U tail Anchor

The A's guide the addition of U's in the mRNA

mRNA AGCAGCGACUUAGCAGCGAC

gRNA UUUUUUCUGAAUCGUCGCUG

Oligo U tail Anchor

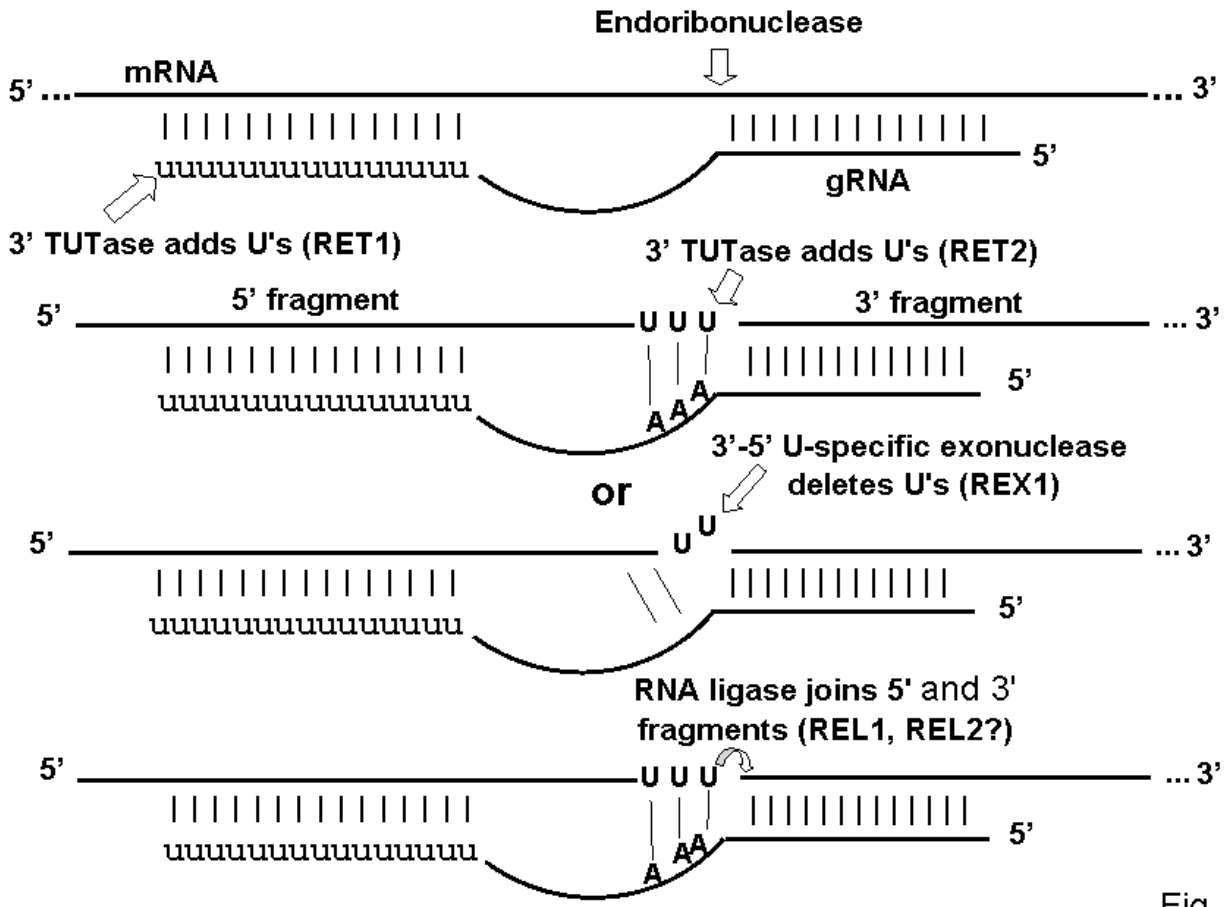
mRNA AGCAGCGACUUAGCAGCGAC

gRNA UUUUUUCUGGGUCGUCGCUG

Oligo U tail Anchor

Remember: G's can do it too

Mechanism of U-insertion/deletion RNA editing



Step 1. Cut

(RNA endonuclease)

Step 2a. U insertion

(TUTase)

OR

Step 2b. U deletion

(RNA Exonuclease)

Followed by

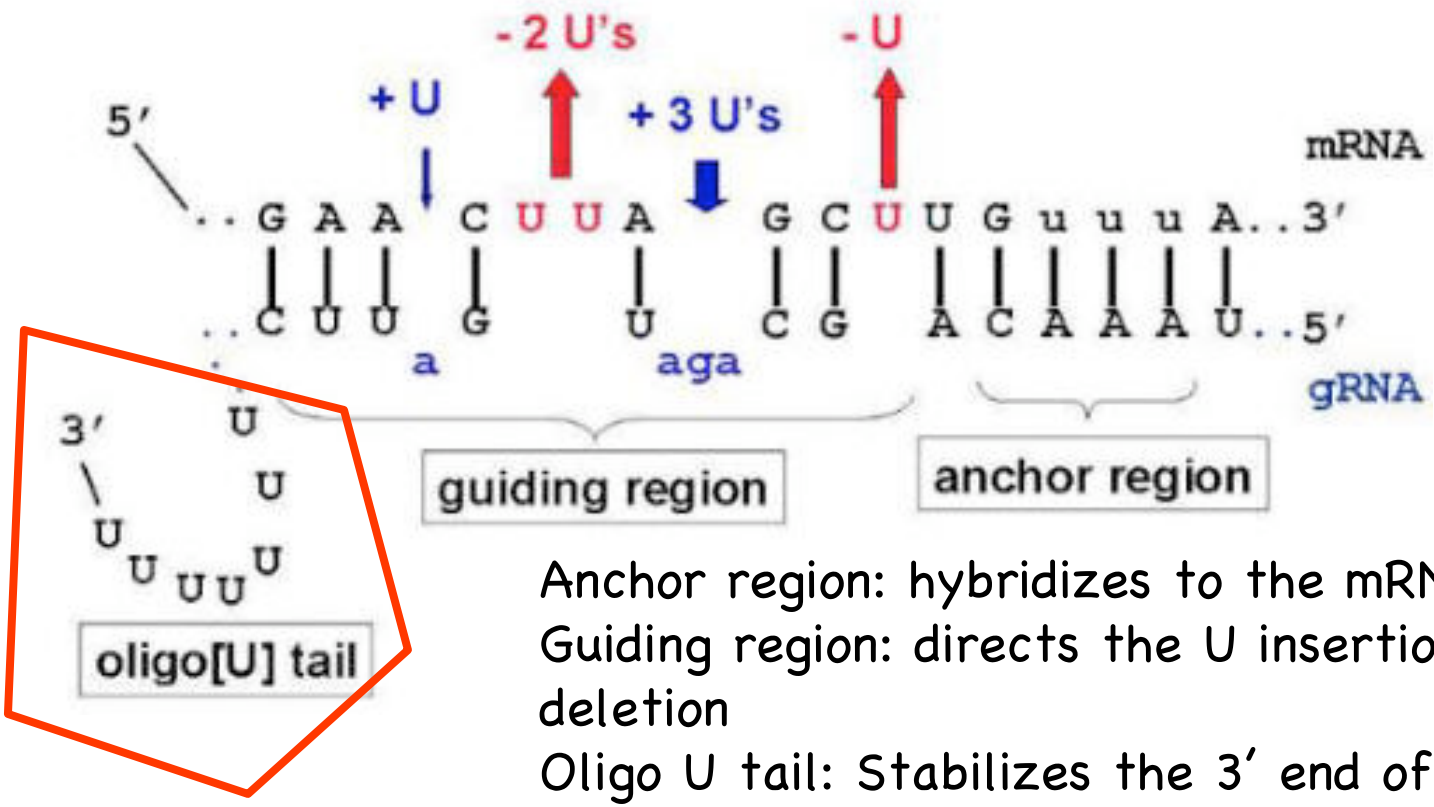
Step 3. Ligation

(RNA Ligase)

Fig. 1

Any editing complex must have enzymes that accomplish these steps

Get to know you gRNA



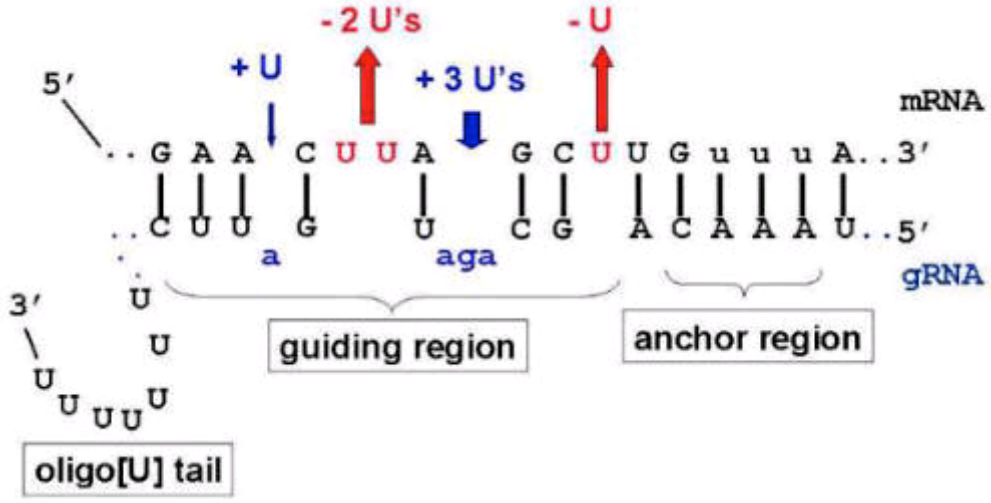
Anchor region: hybridizes to the mRNA
Guiding region: directs the U insertion and deletion
Oligo U tail: Stabilizes the 3′ end of the gRNA.
REMEMBER its added Post transcriptionally!!!

Guide RNA / mRNA interaction

-guide RNA hybridizes to the mRNA at the anchor

-the guiding region contains mismatches
-non-basepaired Us in the mRNA are deleted
-unpaired As and Gs in the gRNA insert Us in the mRNA

-oligo(U) tail is NOT the source of Us for insertion



Misc. guide RNA stuff:	
	oligo U tail varies in length
	~100 different guide RNAs
	one guide RNA per minicircle “species”
	~10,000 minicircles per kDNA network
	multiple copies of each minicircle “species”
	there are also maxicircle guide RNAs

The TAP tagged fusion protein

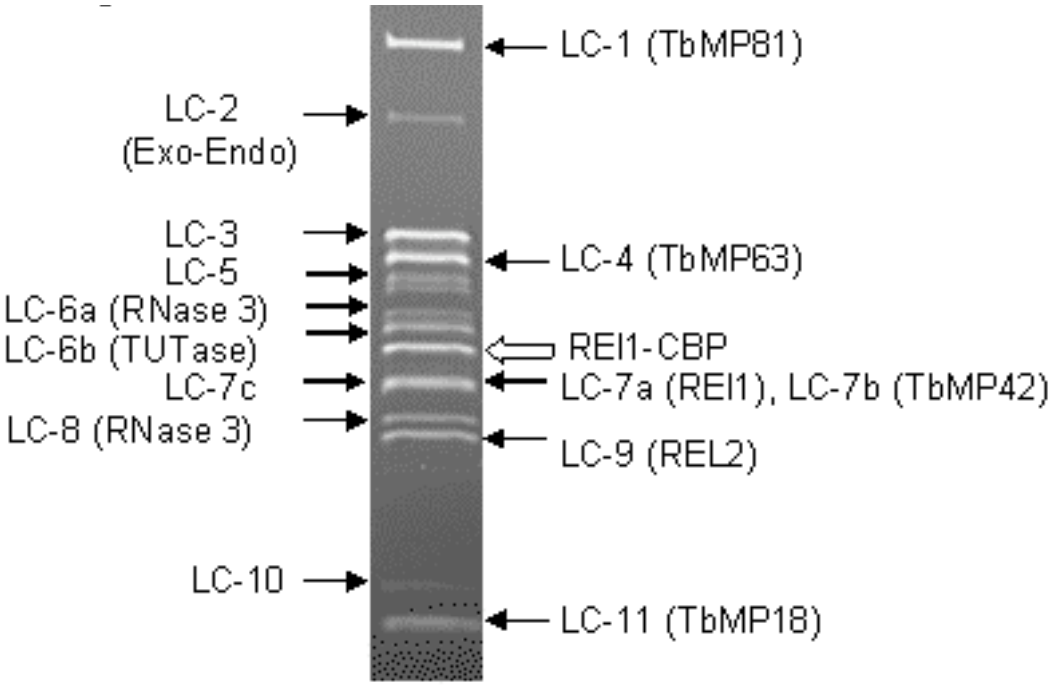
LtREL1 – Leishmania *tarentolae* RNA Editing Ligase 1
CBP – calmodulin binding protein
TEV - tobacco etch virus protease
Protein A – binds IgG (sepharose conjugated)

-the fusion protein is expressed in Leishmania
-mitochondria is isolated from the rest of the cell
-mito lysate is prepared
-fusion protein is bound to an IgG sepharose column
-any proteins associated with Lt REL1 will remain bound to REL!
-TEV protease treatment releases the CBP fusion

-CBP fusion is bound to a calmodulin agarose column
-EGTA to elute from calmodulin column

All the Activities in the Model are Represented in the Tandem Affinity Purification isolated L-complex

RET2 TUTase
3 zf proteins
2 RNase III proteins
2 RNA-binding proteins
REL1- RNA ligase
REL2- RNA ligase
LC-2 or REX1-exonuclease
LC-3 or REX2-exonuclease



L-complex = Ligase containing complex
(recall that the REL1 ligase was used in the TAP isolation)

Editing within a multiple guide RNA Domain Proceeds 3' to 5'

Editing by gRNA 1 creates the correct anchor for gRNA 2
Editing by gRNA 2 creates the correct anchor for gRNA 3
and so on…