Two peas in a pod?

The roles of REL1 and REL2 in uridine insertion/deletion RNA editing

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① Introduction

- Uridine insertion/deletion RNA editing is unique to kinetoplastids and essential for the expression of their mitochondrial genome[1].
- The RNA editing core complex (RECC) performs key catalytic steps, including ligation of edited RNA fragments by the **RNA editing ligases**, REL1 and REL2.
- REL1 is essential for the survival of *T. brucei*[3] but REL2 knockdown has not produced any detectable phenotype[4-5] → suggests functional specialization

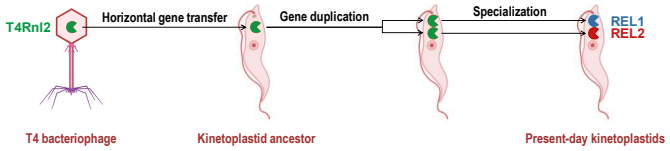

Fig 1. Putative phylogenetic origin of REL1 and REL2. T4 RNA ligase 2 (T4Rnl2) was possibly acquired by horizontal gene transfer *via* bacteria within the arthropod vector[2].

What are the respective roles of REL1 and REL2 in RNA editing?

② Unligated transcripts accumulate upon REL1 repression

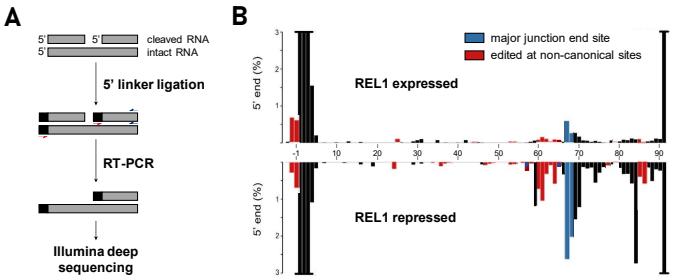

Fig 2. RNA-seq analysis of ND7 5' editing domain upon conditional REL1 expression. (A) Experimental approach to capture RNA editing substrates, intermediates and products. **(B)** Percentage of reads ending at any given nucleotide position along the ND7 sequence in bloodstream form *T. brucei* with REL1 expressed *vs* repressed.

1) Accumulation of transcripts cleaved at sites where editing usually stalls[6]
2) Accumulation of transcripts cleaved erroneously → **Potential repair function**

③ Divergent regions in REL1 and REL2 are predicted to bind RNA

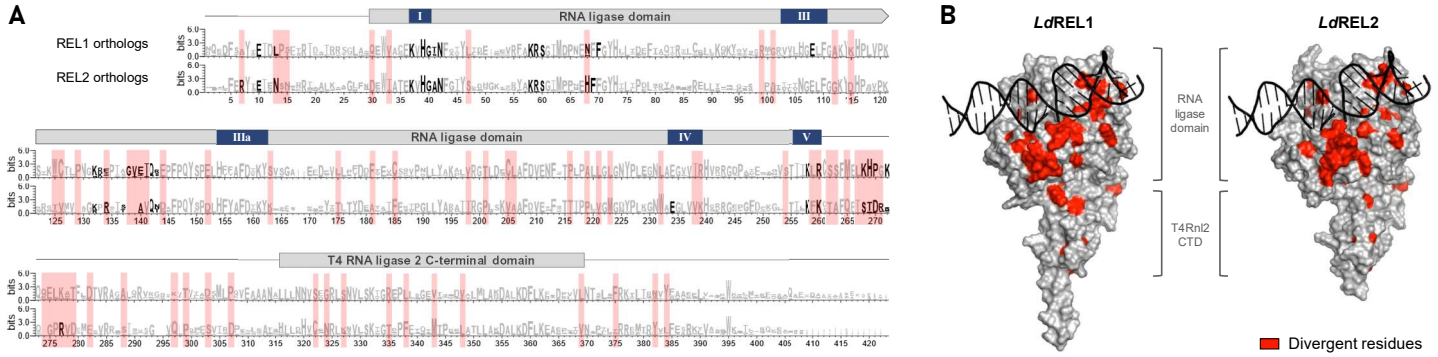

Fig 3. Comparative functional analysis of REL1 and REL2 at the sequence and structural levels. (A) Sequence consensus of REL1 and REL2 orthologs. Divergent residues (red) were determined with multi-Harmony[7]. Conserved protein domains (grey) and ligase signature motifs[8] (blue) are shown above the sequences. **(B)** Structures of REL1 and REL2 from *Leishmania donovani* predicted by DiscobaAlphaFold2[9]. RNA molecules from T4Rnl2 crystal structure (PDB: 2HVQ) were docked onto the models using the HADDOCK web server[10]. Residues in close proximity to the RNA molecules (< 4 Å) are shown in black in panel (A).

④ REL1 has more flexible RNA specificity than REL2

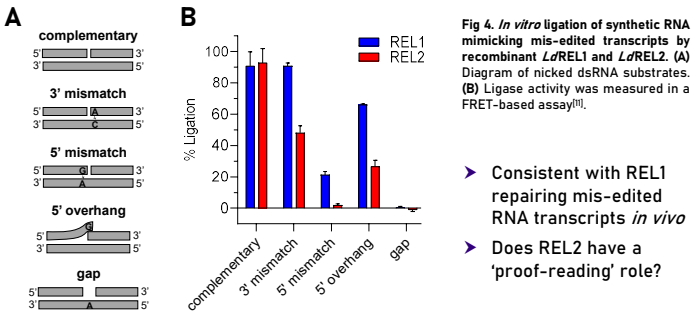

Fig 4. *In vitro* ligation of synthetic RNA mimicking mis-edited transcripts by recombinant *Ld*REL1 and *Ld*REL2. (A) Diagram of nicked dsRNA substrates. **(B)** Ligase activity was measured in a FRET-based assay[11].

- Consistent with REL1 repairing mis-edited RNA transcripts *in vivo*
- Does REL2 have a 'proof-reading' role?

⑤ REL1 is essential in *Leishmania mexicana* but not REL2

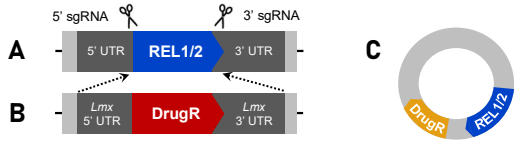

Fig 5. LeishGEdit CRISPR/Cas9 system[12]. (A) Single guide RNAs specify sites for Cas9 to introduce double-stranded breaks in the target genes. **(B)** Drug resistance cassettes replace the regions removed by Cas9 in each allele. **(C)** If REL1 or REL2 is essential, only cells previously transfected with a plasmid expressing an ectopic copy of the gene survive.

Fig 6. PCR to confirm REL1 and REL2 gene knockout (KO). Primers annealed to the target gene coding sequence and 3' UTR to amplify genomic sequences.

- REL1 knockout was only achieved in the presence of an ectopic copy of the gene. In contrast, REL2 null mutants were viable.
- **Strong evidence that REL1 is essential in *L. mexicana* promastigotes but not REL2.** Consistent with prior findings in *T. brucei*[3-5].

⑥ What's next?

- Mutagenesis analysis to confirm the role of divergent regions in RNA binding
- Optimising *Ld*REL1 and *Ld*REL2 crystallisation conditions
- Evaluating RNA specificity of RECCs with catalytically dead REL1 *vs* REL2
- Deep sequencing of mitochondrial mRNAs in *L. mexicana* REL2 null mutants

References

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