Rat KRAB-ZNF genes evolving through tandem duplications: targets of endogenous RNAi in oocytes

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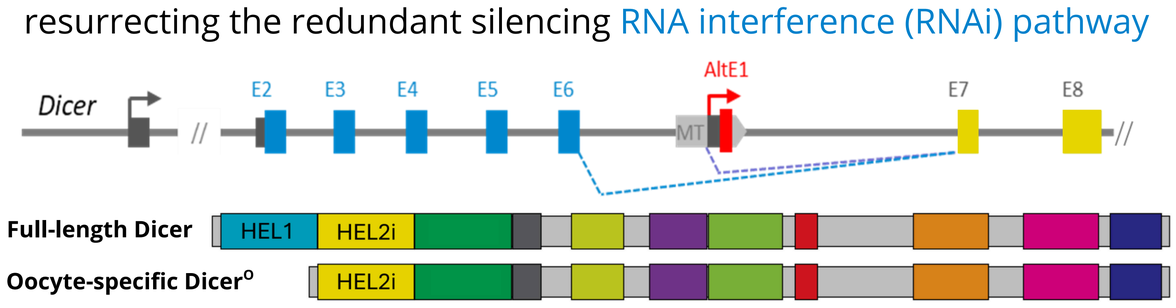
Objective

Functionally characterize selected putative zinc finger protein transcription factors suppressed by RNA interference in rat oocytes

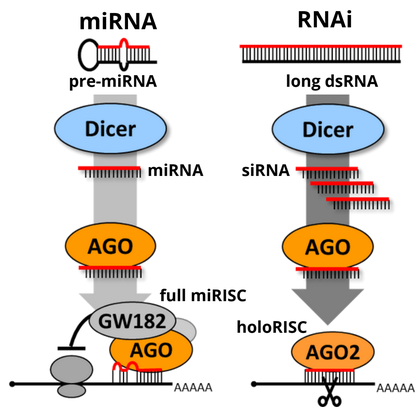
Background

- MT-C retrotransposon LTR insertion, acting as an alternative promoter, drives expression of a shorter isoform of RNase III enzyme Dicer in mouse and rat oocytes
- Truncated oocyte-specific Dicer^{O} is more efficient at siRNA production, resurrecting the redundant silencing RNA interference (RNAi) pathway



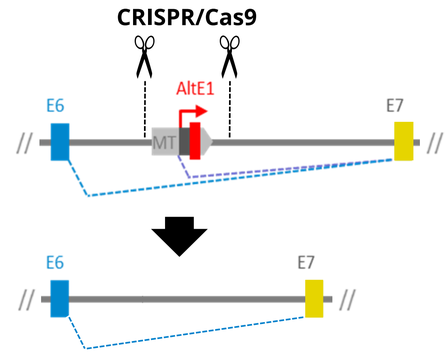
MT-C insertion provides an alternative exon 1 that is spliced directly to exon 7, omitting first six exons of Dicer. Resulting oocyte-specific Dicer^{O} isoform is missing the N-terminal HEL1 domain, allowing it to produce siRNAs efficiently.

- One Dicer enzyme used in both the miRNA and RNAi pathways in mammals
- miRNA pathway, facilitating translational gene regulation, is favoured due to mammalian Dicer's structural adaptation
- RNAi, used for post-transcriptional regulation and antiviral defense, became obsolete in most mammals

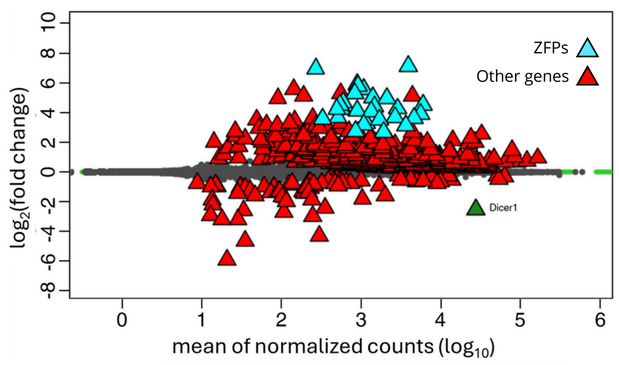


Scheme of two pathways utilizing Dicer. Dicer processes two substrates - pre-miRNAs, cleaved into miRNAs and used in a full silencing complex, or dsRNA, cleaved into siRNAs and loaded onto AGO2, leading to sequence-specific RNA degradation.

- Knock-out of Dicer^{O} promoter in rat reduces RNAi effectivity in oocyte
- As a result, multiple genes get upregulated, including dozens of uncharacterized KRAB-zinc finger protein (KZFP) genes
- KZFPs are known as quickly evolving transcriptional repressors and TE silencers, specifically binding DNA and inducing heterochromatin
- These discovered rat KZFPs have no homologs in mice

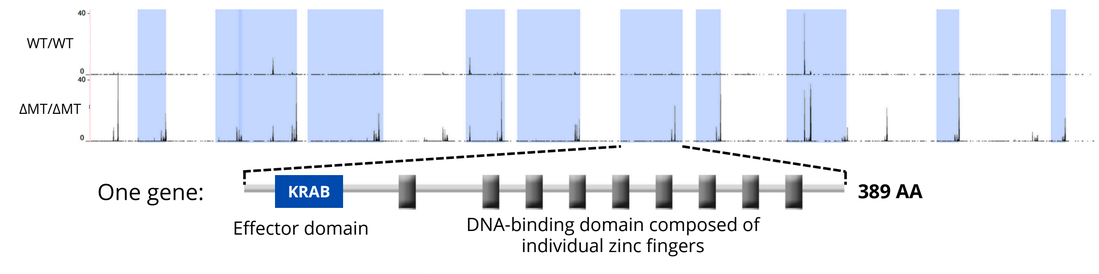


Alternative Dicer^{O} promoter knock-out in rat using CRISPR/Cas9



Differentially expressed genes after Dicer^{O} KO. Zinc finger protein genes are shown in cyan.

- KZFP genes arranged in clusters created by tandem duplication or found individually at the ends of chromosomes
- Number of mapped reads increases several-fold upon Dicer^{O} KO



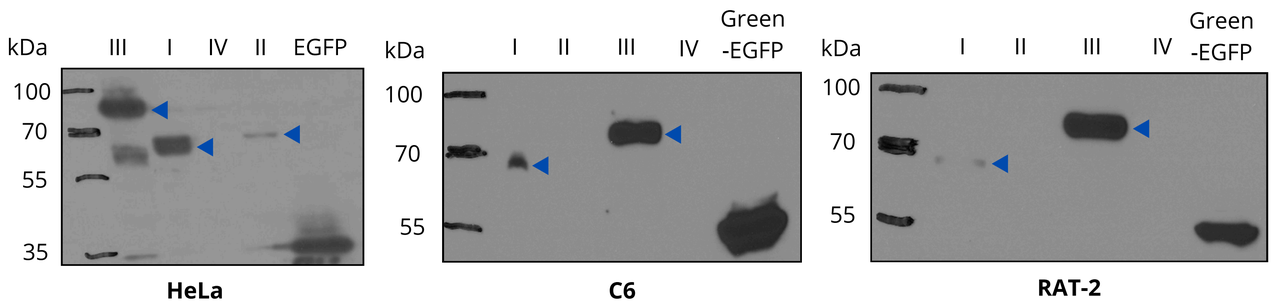
UCSC genome browser screenshot of a cluster of ZFP genes on rat chromosome 9, each highlighted in blue. The two tracks show number of mapped reads from WT and ΔMT/ΔMT rat. Predicted structure of one zinc finger protein is shown below.

Research questions

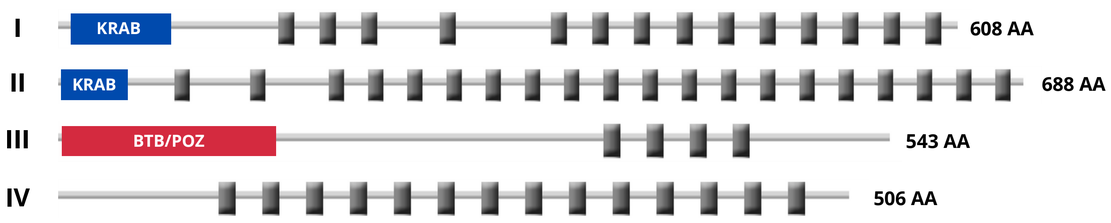
1. Do the selected genes produce stable proteins? Do they localize to the nucleus, as is expected of TFs?
2. Do the proteins each distinctly affect the transcriptome after overexpression in cultured cells?
3. What are the genomic binding sites of these proteins?

Current results

- Four proteins selected based on highest expression level and relative change and cloned into FLAG-tagged expression vectors
- Two with KRAB - most representative of the whole group
- One with BTB/POZ - an orphaned outlier with a different effector domain
- One with no effector domain - lost its KRAB due to a frameshift mutation



Western blot, anti-FLAG. Proteins II and IV not detected in rat cells yet, further protocol optimizations are planned.

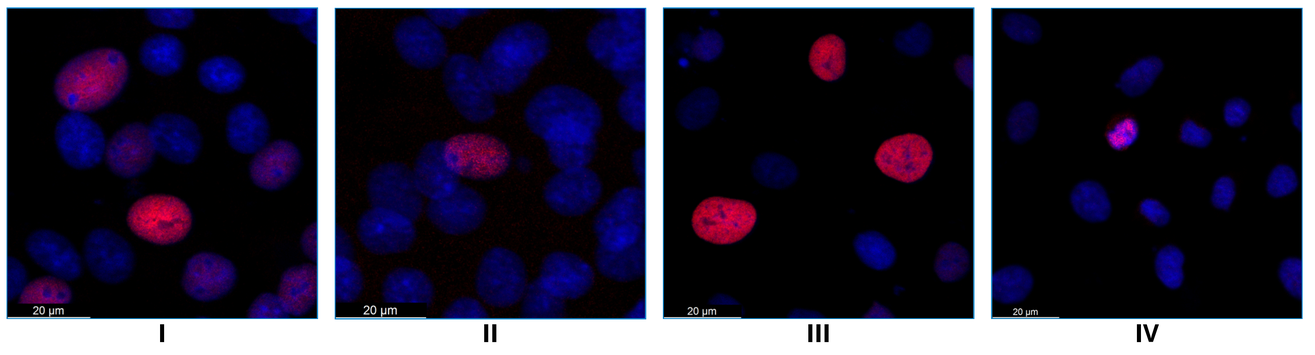


Predicted structures of four proteins selected for further experiments.

- Three zinc finger proteins successfully detected via western blotting upon overexpression
- ZFPs transfected into HeLa, NIH3T3 and 2 rat cell lines - glioma (C6) and embryonal fibroblasts (RAT-2)

- All four zinc finger proteins observed accumulated in the cell nuclei after immunofluorescent staining.

*RAT-2 cells, imaged using confocal microscopy. Stained using **DAPI** and **anti-FLAG+Alexa 594**.*



Summary & plans

- All four selected uncharacterized zinc finger protein genes produce stable proteins, even if their detected levels vary. All four proteins localize strictly to the nuclei of observed mammalian cells. This supports our hypothesis of them functioning as transcription factors.
- To uncover whether these proteins bind to specific genomic sites and affect the transcriptome in distinct ways, we will perform RNA-seq and ChIP-seq experiments.
- We hypothesize that RNA interference suppressing quickly evolving putative transcription factors specifically in oocytes might represent a sort of a "toxin-antidote" genomic conflict, where the toxin in the form of rapidly evolving ZFPs is neutralised by RNAi antidote.

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