Non-coding elements showing accelerated evolution in subterranean mammals drive expression in zebrafish retina

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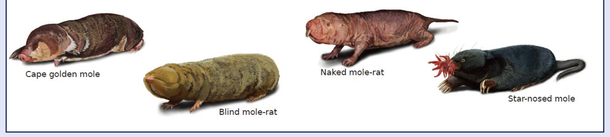
Introduction

- Underground mammals usually have **small or no eyes** in adaptation to their dim-light environment.
- Eye genes and enhancers of underground mammals accumulated mutations at higher rates than their relatives living aboveground, due to **relaxed evolutionary constraint**.
- While the regressive evolution of ocular genes is well described at the gene level, the evolution of their **regulatory DNA** is still unknown.

Aim: Find non-coding elements showing accelerated evolutionary rates in underground mammals, and characterize their expression in zebrafish.

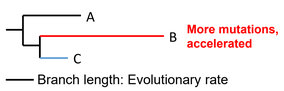


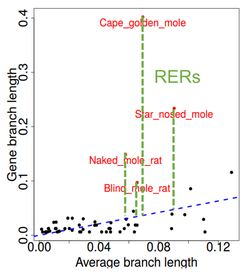
Methods

- Relative evolutionary rates (RERs)

We used comparative genomics method to find out genes and regions with **accelerated evolutionary rates** in 4 blind mole species (cape golden mole, blind mole-rat, naked mole-rat and star nosed mole) than other species.

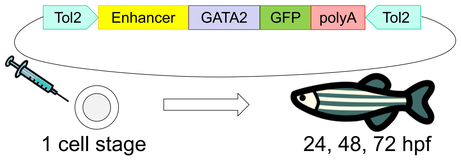
Species A: ATCCGACTACGTACGGCA
Species B: CGACAGGTAGAATAGGCG
Species C: ATCGGACTACGTACGTCA





- Tol2 transposase system

We then construct plasmids containing top mole-accelerated enhancers to drive GFP expression in zebrafish embryos.



Mole-accelerated non-coding elements show overlap with mouse retina specific open chromatin regions

The overlap between mole-accelerated non-coding elements and mouse retina specific open chromatin regions implies **activated transcriptional activity** of those elements in retina.

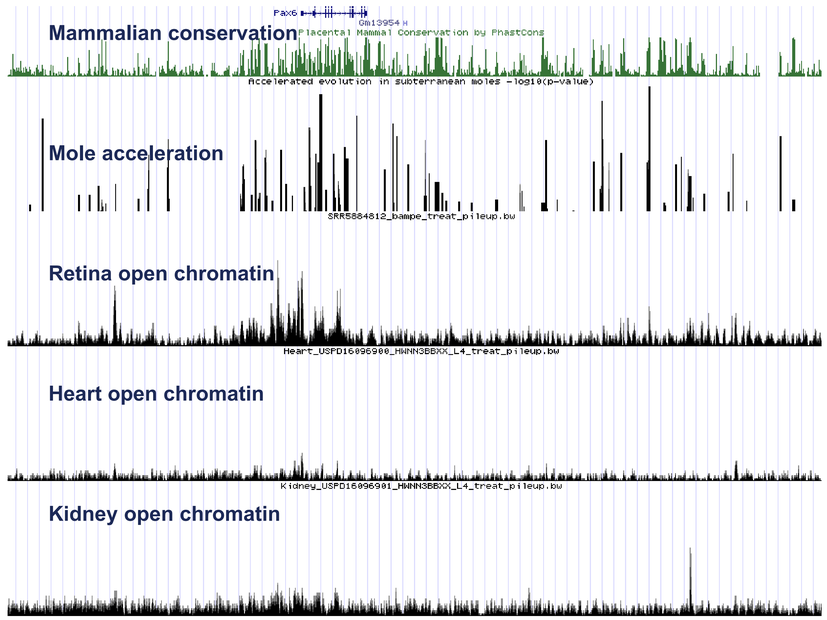


Figure 1. Mole-accelerated non-coding elements (peak height represents –log10(P value)) shown with mammalian PhastCons, mouse retina, heart and kidney ATAC-seq results in UCSC genome browser, around Pax6 gene region. (Data source: Retina - SRR5884812)

Top accelerated cis-regulatory regions (CREs) out of a genome wide scan of 340,000 regions

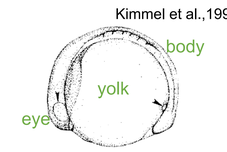
We selected top 5 cis-regulatory regions accelerated in moles to characterize, and 2 retina enhancers around Pax6 gene as positive controls. In these CREs, cre4 is near Sox2, an essential developmental gene in neurogenesis, while other CREs' neighbor genes are not well known for their eye-related functions.

Table 1. Cis-regulatory regions (CREs) tested in zebrafish (2 positive controls and top 5 mole-accelerated regions)

Name		Coordinates (hg19 genome)				Neighbor Gene	
		chr	start	end	length	Symbol	Full Name
alphaR (positive control)	alphaR_enh	chr11	31825492	31826716	1225	Pax6	paired box 6
HS23R (positive control)	HS23R_enh	chr11	31676829	31677932	1104	Pax6	paired box 6
cre1	chr6cre10170	chr6	91074960	91075001	42	Bach2	BTB and CNC homology, basic leucine zipper transcription factor 2
cre2	chrXcre6057	chrX	85602548	85602724	177	Dach2	dachshund family transcription factor 2
cre3	chrXcre9929	chrX	129136241	129136285	45	Bcorl1	BCL6 co-repressor-like 1
cre4	chr3cre24081	chr3	180816611	180816730	120	Sox2	SOX2 overlapping transcript (non-protein coding)
cre5	chr9cre1979	chr9	13108812	13108893	82	Tyrp1	Tyrosinase Related Protein 1
...							...

4 out of top 5 accelerated CREs show expression in developing zebrafish neural system (including retina)

A high proportion of top mole-accelerated CREs drive GFP expression in developing neural system of zebrafish, including retina.
We anticipate mosaic pattern of GFP expression in the first generation of transgenic zebrafish because of the limited efficiency of Tol2 transposase.



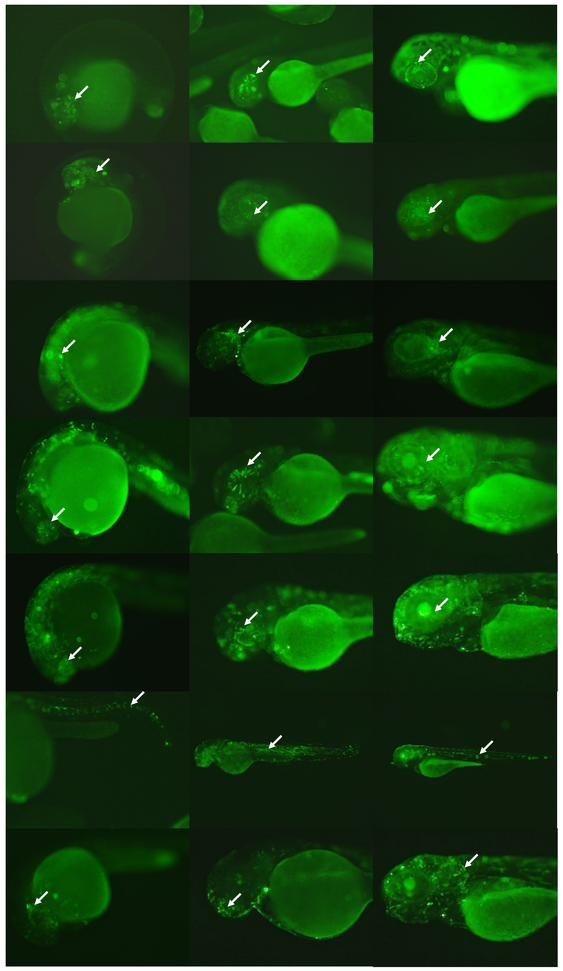


Figure 2. Mole-accelerated CREs driven GFP expression in zebrafish embryos

Future plans

- Compare open chromatin regions in retina of guinea pig and naked mole-rat;
- Build a zebrafish line to achieve stable enhancer-GFP expression without mosaic pattern;
- Characterize mole-accelerated enhancer-GFP expression in neonatal mice using adeno-associated virus (AAV).