Can human genes inhibit LINE retrotransposition on zebrafish?

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Abstract

Transposons are mobile pieces of DNA that can jump to different places of the genome. LINEs are a type of Transposable Element that move by a copy and paste mechanism using a Reverse Transcriptase activity. LINEs are very abundant in genomes, including our genome and the genome of the model organism *Danio rerio* (zebrafish). Because of their mobility, they can create mutations, as they can insert themselves in genes. This can be either beneficial, neutral or harmful for the organism, which is why we want to study how the genome control their activity.

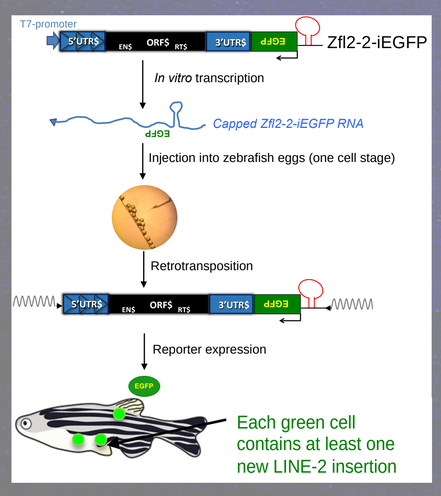
There are some genes that can inhibit LINE retrotransposons, as the Apobec3A gene (A3A), which is present only in the genome of hominids. Since it is not present in the zebrafish genome, we can use this model organism to try to understand its efficiency and mechanism of inhibition *in vivo*.

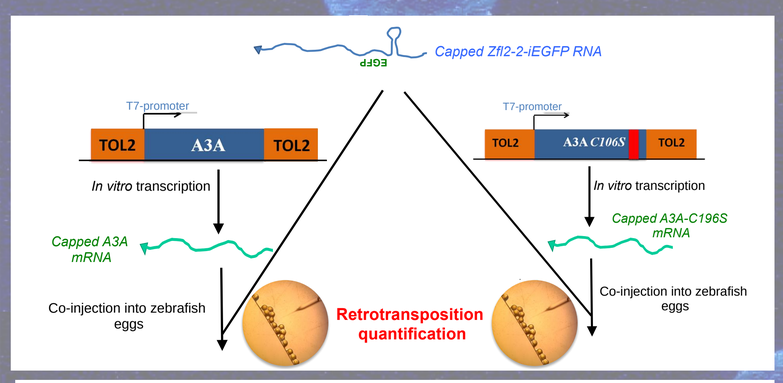
To do that, we used an *in vivo* LINE-EGFP mobility assay by injecting RNAs into zebrafish embryos, together with the RNA encoding for human A3A or a mutant control. Remarkably, our results indicate that A3A can inhibit the mobility of zebrafish LINEs, suggesting that A3A is a generic inhibitor of LINE retrotransposition.

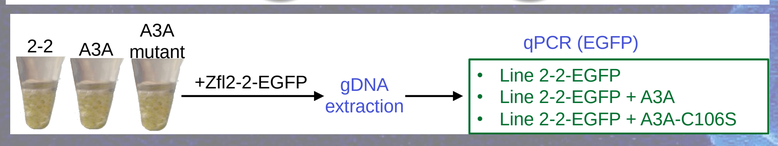
Hypothesis

Previous studies have shown that the human A3A gene can inhibit human LINE-1 retrotransposition *in vitro*. To test whether A3A could inhibit other LINE retroelements, in this study we tested if human A3A could inhibit the mobility of zebrafish LINEs, using an *in vivo* assay.

Methods





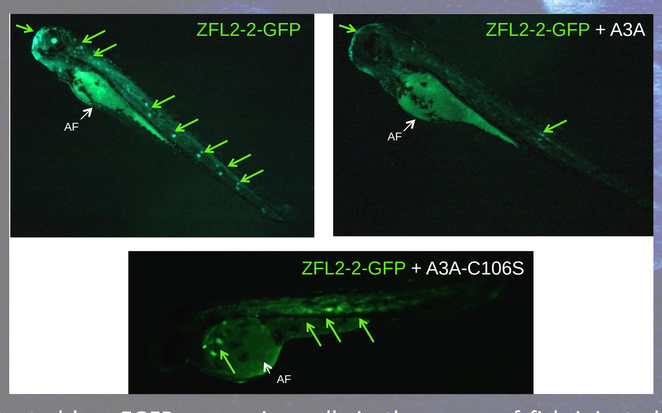


Results

We divided the zebrafish fish eggs into three groups. Each group was injected with the following types of *in vitro* transcribed RNAs:

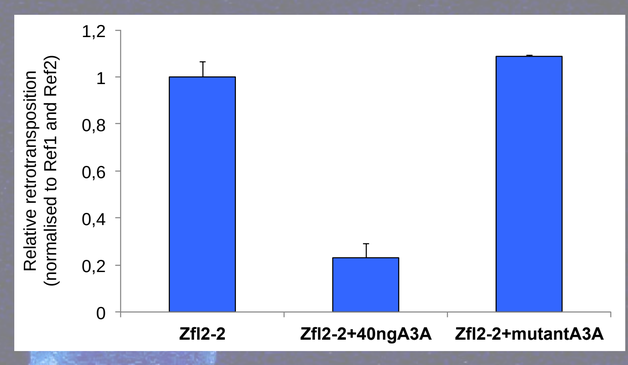
- Line 2-2-EGFP (the active retrotransposon with the EGFP reporter gene)
- Line 2-2-EGFP + A3A (with human A3A RNA)
- Line 2-2-EGFP +A3A-C106S (with mutant human A3A RNA)

Below are shown results from the assay.



On the pictures, we detected less EGFP-expressing cells in the group of fish injected with the A3A RNA. Thus, these data indicate that A3A can inhibit LINE-2 mobility in zebrafish.

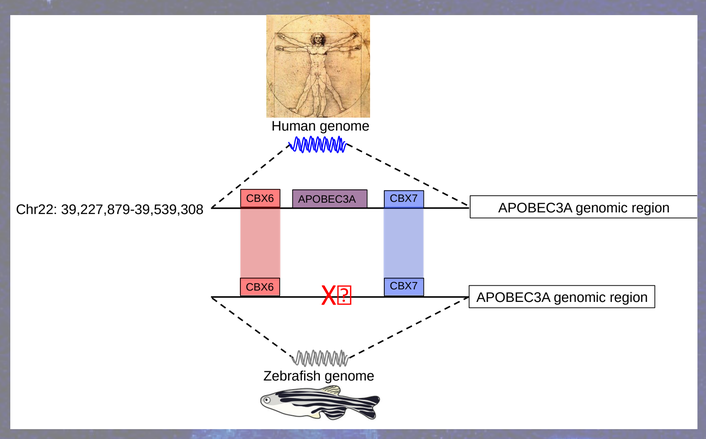
To quantify the level of LINE-2 mobility *in vivo*, we extracted genomic DNA from the three groups of injected zebrafish embryos and conducted a quantitative PCR assay where we determined the number of EGFP inserted copies. In this assay, each copy of EGFP correspond to an insertion.



As expected, the graph confirmed that the fish injected with A3A had the lower number of EGFP copies. Indeed, there are 5-times less LINE-2 insertions in the presence of A3A. As a control, the mutant A3A RNA had no effect on the copy number of EGFP insertions.

Discussion

The gene APOBEC3A inhibits retrotransposition in human cells and also when injected into zebrafish embryos. The A3A gene is not present in the zebrafish genome, even if our genome and the zebrafish genomes are 40% identical.



The lack of the A3A gene in zebrafish may mean that zebrafish normally accumulate more LINE insertions than humans.

Another important aspect of our study is the demonstration that A3A can efficiently inhibit LINE retrotransposition *in vivo*. Thus, we plan to generate transgenic zebrafish expressing A3A in order to reduce LINE mobility *in vivo* and to test the effect of LINE mobility in the zebrafish brain.

Conclusions

- The human APOBEC3A gene is able to inhibit the mobility of zebrafish LINE-2 elements in an *in vivo* mobilization assay.
- The inhibition of LINE-2 retrotransposition by A3A requires its Cytidine Deaminase activity, as the mutant A3A is not able to inhibit LINE mobilization.

Acknowledgements

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