Epigenetic engineering of *Schistosoma* vector snails

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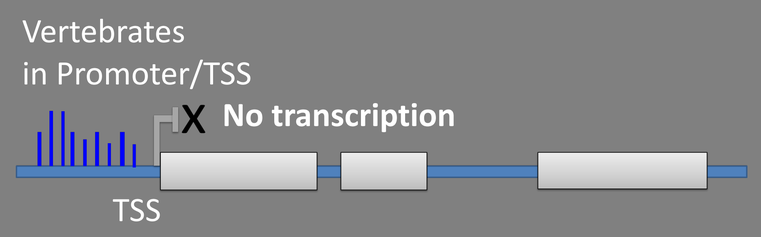
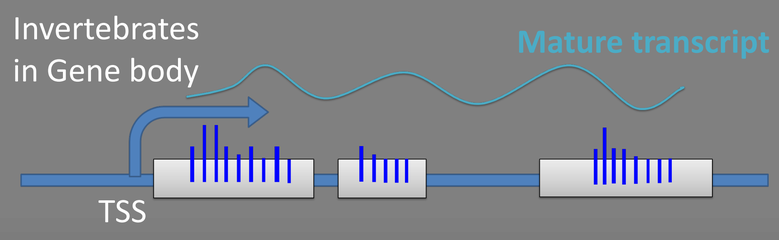
Context

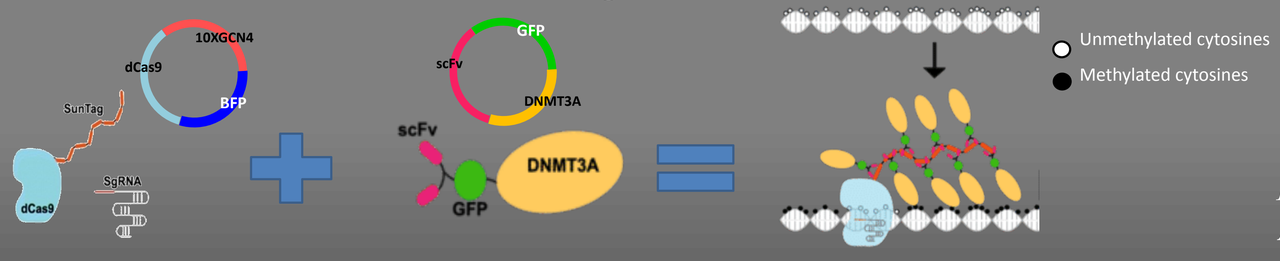
Biomphalaria glabrata is the mollusk intermediate host for the parasite *Schistosoma mansoni*, causal pathogen of schistosomiasis. Approximately 200 million people in 74 countries suffer from schistosomiasis, being the most severe tropical disease after malaria in terms of morbidity. Phenotypic variations are important in host-parasite interactions in which both selective pressure and rate of evolution are high and epigenetic changes are expected to be drivers of phenotypic plasticity.





B. glabrata exhibits intragenic methylation predominantly in exons. Although promoter DNA methylation is an important regulatory component of vertebrate gene expression, its role in invertebrate gene regulation has been little explored. Instead, gene body DNA methylation (GBM) is associated with expression of invertebrate genes, but its role remains enigmatic.

Objective

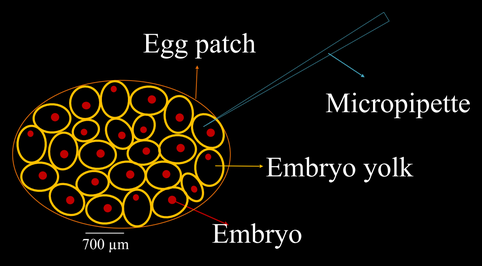


Conduct targeted DNA methylation with a nuclease-deactivated Cas9 protein fused to repetitive peptide epitopes (SunTag) recruiting multiple copies of antibody-fused DNMT3A and determine the molecular phenotype of this targeted methylation.

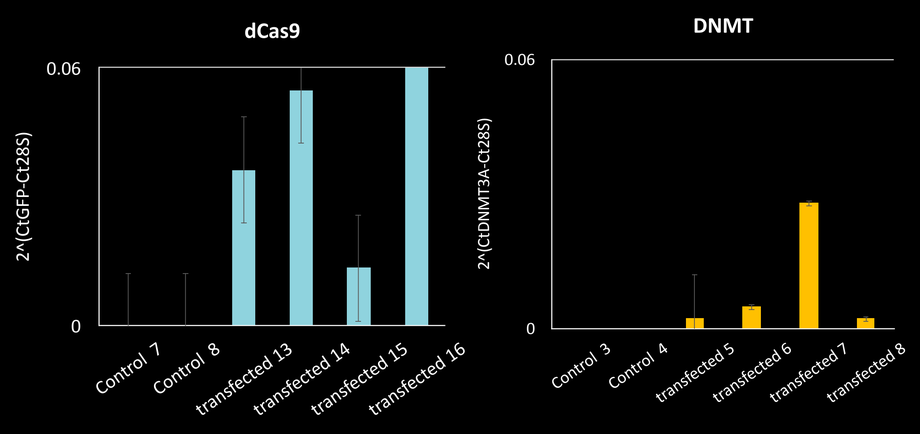
Results

In vivo transfection technique stablished : Microinjection of plasmids in embryo yolk sac at gastrula stage with an *in vivo* polymer based reagent .

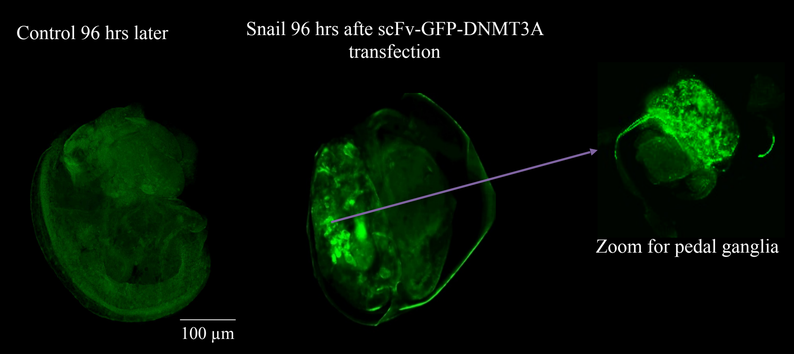
Microinjection technique shows an efficiency of 2-4%
Video guide on:



Validation of transcription of dCas9 and DNMT3A by RT-qPCR.

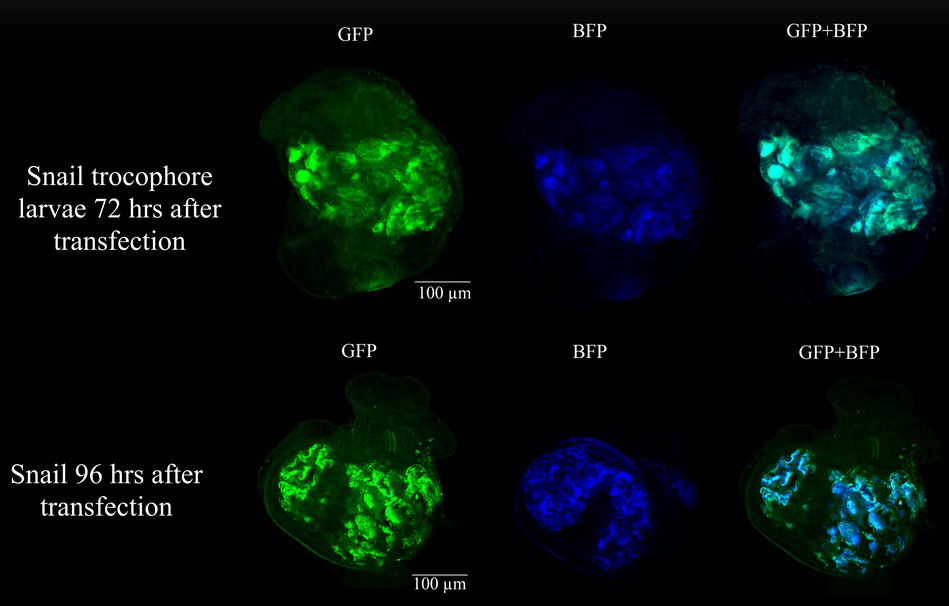


Validation of protein expression by fluorescense microscopie.

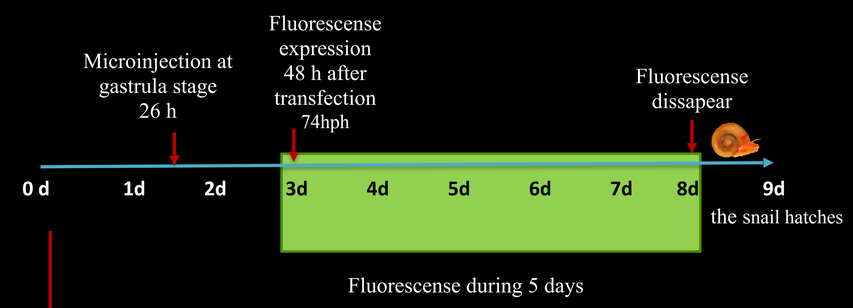


Co-transfection of plasmids scFv-GFP-DNMT3A & dCas9-SunTag

In co-transfected snail embryos, green marking is more important than blue one, coherent with the SunTag system of the plasmid that recruit multiple GFP's



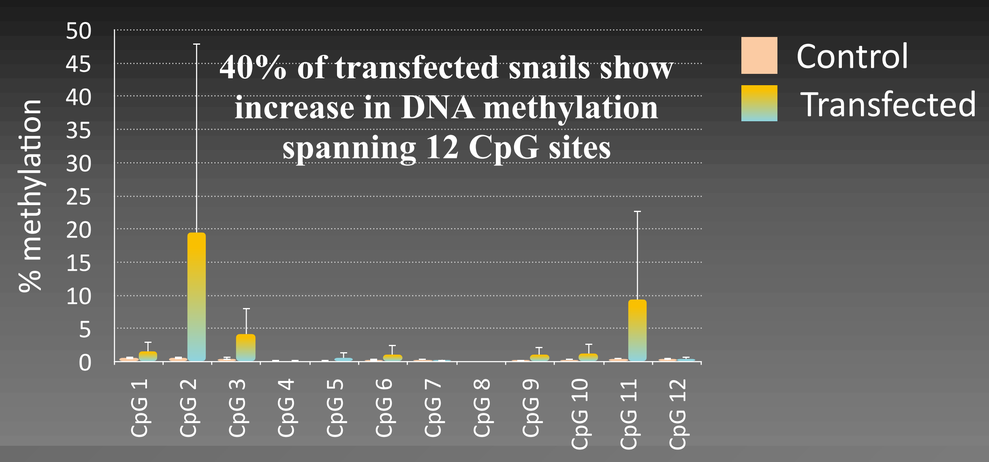
Plasmids containing a SV40 promoter and showing a transient expression during 5 days



Time window to perform targeted hit-and-run DNA methylation

Take home message

Based on an established system for human cell lines, we have put into place a tool to perform targeted DNA methylation in the snail *B. glabrata*. We are now ready to investigate the causal relations between invertebrate gene-body DNA methylation, gene expression and phenotypic change.



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