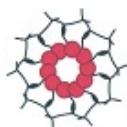
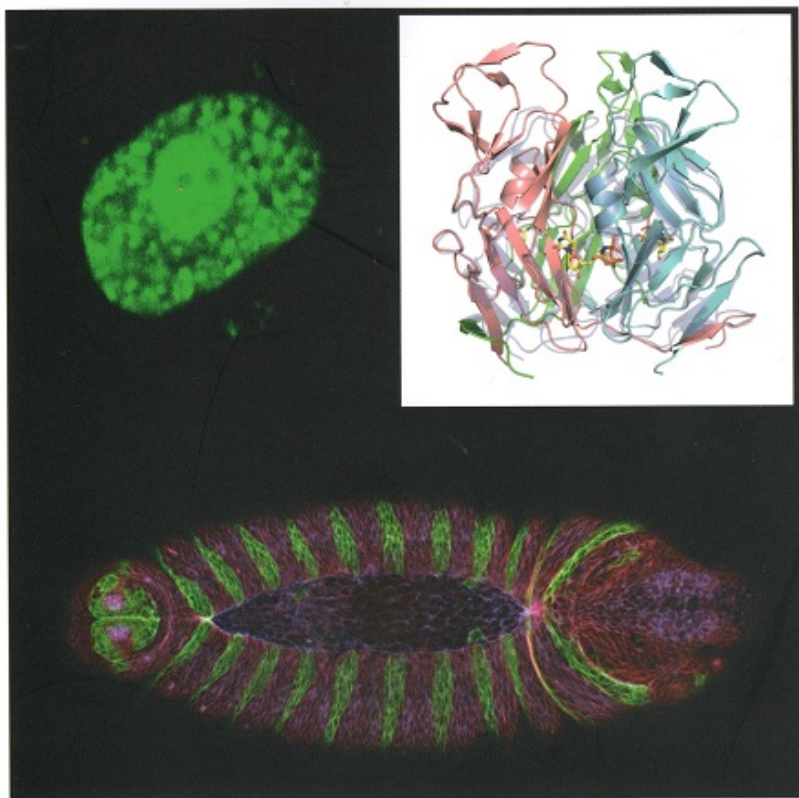


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inducible expression plasmids were also constructed. In summary, the CRISPR/Cas9 tool-box created by us will be valuable for future work to perform targeted gene modifications in *N. benthamiana*.

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IDENTIFICATION AND EXAMINATION OF RABBIT MICRORNA-S IN DIFFERENT DEVELOPMENTAL STAGES OF EMBRYOS AND STEM CELLS

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MicroRNAs (miRNA) are ~22nt long, non-coding RNAs, which have important regulatory role in several fundamental biological processes by posttranscriptional targeting the 3' UTR of mRNAs causing translational repression or the degradation of the mRNA. They are very important in maintenance of the pluripotency and promoting cell differentiation.

Our aim is to establish a real embryonic stem cell line from rabbit, to obtain germline chimaeras. With this goal we started to investigate which miRNAs can be found in different developmental stages of rabbit embryos and in stem-like cells. It would be important to understand their role in embryo development and to know which kind of modifications would be necessary to maintain the pluripotency of the stem-like cells.

Because of no published rabbit miRNA sequences was available at the beginning of our study, samples derived from different stages of rabbit embryos and stem cells were sequenced by using SOLiD platform. The results were checked for new rabbit miRNA sequences according to known human, mouse and bovine miRNAs.

1693 new rabbit sequences were identified. From these sequences three clusters were chosen to further analysis, known to be stem-cell specific in mouse and human, and some extra miRNA known to have important role in early differentiation. We compared two different methods to RNA isolation based on RNA purity and integrity. We tested the hypothesis whether the read numbers getting by SOLiD sequencing are suitable for predicting the gene expression level.

We could identify where these miRNAs are localised in the rabbit genome, and clarified the structures of these clusters. First we measured the expression of selected miRNAs in 6 rabbit tissues or embryo derived samples by real-time PCR, then in 6 day embryo, epiblast, hypoblast and trophoblast cells. We found that the *ocu-miR-290* and *ocu-miR-302* clusters are stem cell specific, but no specificity was found regarding the *ocu-miR-17-92* cluster. Not surprisingly, PGCs are really similar to ESCs, while iPSCs express the pluripotency markers at a very high level, what clearly shows the success of the reprogramming.

At last, we examined the expression of the *miR-191* and *miR-302* miRNAs in 6-day embryo by *in situ* hybridization. We found that the *miR-302* show higher expression in embryoblast than in trophoblast, which is consistent with our real-time PCR data.

Keywords: *microRNA, regulatory RNAs, rabbit, stem cell*