Notes

Methods

Whole genome sequencing -> PacBio, along with Illumina for error correction in 1% of contig bases

Development of a new genome assembler, due to the large genome size (x10 human size) -> MARVEL

Verification of the genome assembly using non-exonic ultraconserved elements (UCEs) and generation of a gene catalogue using mRNA to determine if these coded for conserved eukaryotic genes

Previously developed molecular toolkit -> germline transgenesis, CRISPR gene mutation, viral transfection... -> Identification of the regeneration cells

Findings

The median intron size is quite large (12-17 times compared to humans) -> Smaller intron sizes in non-developmental genes, which could facilitate rapid transcription and upregulation

- Pax family of genes:

Presence	Absence
Pax10	Pax7
	Pax3

Pax3 and its cis-regulatory elements seem to be absent due to a deletion, although experiments performed using CRISPR mutate the gene show that Pax7 performs the functions of Pax3 along with the usual Pax7 functions in axolotl

Limb regeneration

Use of published mRNA and miRNA transcripts and the authors' own transcriptional profiling -> identification of 5 upregulated transcripts

Protein	Function
similar to tectorin	Extracellular matrix
Ly6	uPAR surface receptor

Mapping non-coding RNAs -> 93 pre-miRNA, 42 novel miRNAs

Future work

Model organism to study the evolutionary basis of its regeneration ability

Questions

Why was (were) this (these) genome(s) sequenced in the first place?

Because the axolotl is a very insteresting model organism to study the molecular and evolutionary aspects of its regeneration ability. This requires the analysis of its genome regulation and evolution.

What makes this paper interesting from a technical point of view? Where there technical challenges that needed to be overcome? What was the biggest technical challenge or hurdle?

The authors were able to sequence the huge genome (32 Gb), which is approximately 10 times the human genome size, and contains a large number of repetitive sequences (65.6%, roughly 18.6 Gb), most of which were long terminal repeats which could span more than 10 kb in length. In order to achieve this, the authors used PacBio reads alongside Illumina reads (to correct errors in contig bases). Furthermore, they developed a novel assembler to assemble large genomes.

Find (an) example(s) of 'nothing makes sense in biology except in the light of evolution'. In other words, focus on one or more examples of how evolutionary adaptation is reflected in the genome (if possible).

The genome contains a large number of long terminal repeat retroelement classes and endogenous retroviruses, which seems to indicate that the axolotl went through a long period of transposon activity followed by a recent burst of

expansion. Furthermore, the axolotl also presents a high median intron size (22kb roughly 13 times bigger than humans), although this median size expansion is significantly lower in developmental genes (which is the opposite in humans) and seems to suggest that these smaller genes facilitate a rapid upregulation in development.

What 'key' insight(s) did you get from the paper? In other words, imagine you have to tell your friends or family: 'did you know that ... (amazing finding from genome paper)'?

The authors of this paper were able to sequence a very large genome, with a big number of repetitive regions, which is a big technical challenge, and was almost infeasible until the advent of third generation sequencing techniques. The sequencing had a very high read accuracy (99.2%), which was assesed using multiple techniques from comparative genomics.

Why do you think this paper is – or could be - published in a top tier scientific journal?

This scientific article was published in a top tier journal, in this case Nature, because its authors were able to first sequence an extremely challenging organism de novo with a very high read accuracy (99.2%), assemble its reads using an in-house developed genome assembler (MARVEL), a thorough assesment of the completeness of the assembly (using ultraconserved elements and transcriptome analysis), the annotation process of protein-coding genes and knock-out experiments in order to determine the effect that a gene deletion (Pax3) had on the development of the organism. The work that the authors performed pave the way for new discoveries of the intricacies of the axolotl's regeneration abilities.