The axolotl genome and the evolution of key tissue formation regulators

Introduction

Axotlotl, or Ambystoma mexicanum is a type of salamander that is characteristic because it does not undergo metaphormosis, in contrast to most amphibians. They conserve features from salamander larvae such as gills and remain in an aquatic environment. They have been an organism of interest in scientific research due to their impressive regeneration abilities that even allow them to regenerate neural tissue.

Genome characteristics

Even though the axolotl was identified as a good model organism a long time ago, sequencing its DNA was not possible until very recently due to its very large genome size of 32 Gb, approximately 10 times bigger than the human genome and the high number of repeats, which amount to 18.6 Gb roughly 65% of the genome size. Most of these repeats are long terminal repeats (LTR) which occur in retrotransposons and endogenous viruses, this seems to indicate that the axolotl went through a long period of transposon activity. Some of these LTRs span more than 10 kb in length, which is an additional sequencing challenge.

Methods

The authors used the Pacific Biosciences platform, which is a third generation sequencing method that allows the sequencing of very long reads. In addition, Illumina sequencing was used in order to correct sequencing errors in contig bases. This process yielded a very high accuracy of 99.2%. A novel assembly algorithm was developed to deal with the long reads of PacBio and to add a read-correction step, in order to achieve the high accuracy previously mentioned. A thorough assessment of the completeness of the genome assembly was performed using ultraconserved elements and by aligning transcripts from multiple tissues to the genome, of which 85% aligned to the genome.

Genome annotation and analysis

After the sequencing, the authors were able to annotate the genome. They found aproximately 23.000 genes, which is similar to other vertebrates. One of the striking characteristics of the axolotl genome is the high median intron size, which is roughly 13 times bigger than humans. This median size expansion is significantly lower in developmental genes (in contrast to humans, mice and frogs) and seems to suggest that these smaller genes facilitate a rapid upregulation in development. Furthermore, 5 upregulated transcripts were identified in the limb blastema (which is the tissue involved in regeneration), one of the proteins is similar to tectorin (a component of the extracellular matrix) and a Ly6 family member, which have been identified in other salamander studies as playing a role in regeneration.

Comparative genomics analysis

Finally, the authors decided to analyze homologs of a the Pax-family of genes, which is involved in the development of animals. Certain members of the family were missing, such as Pax4 and Pax3. Pax4 is usually not found in amphibians, but the deletion of Pax3 raised some questions. The authors found that Pax7 in the axolotl contains the functionality of Pax3, in order to verify this claim individuals with a mutated Pax7 genome were obtained using CRISPR and TALEN, 2 genome editing techniques. As we can observe on the figure, these mutants had a delay in muscle growth and could not maintain and erect posture, which validated the claim.

Conclusion

The authors of this study were able to 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, the annotation process of protein-coding genes, the experimental validation of the role of the Pax7 gen and the identification of transcripts associated with limb regeneration. The work that the authors performed pave the way for new discoveries of the intricacies of the axolotl's regeneration abilities.