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The Only Clear-Blooded Vertebrate: The Antarctic Icefish

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ABSTRACT

Today, the Channichthyidae are regarded to be one of the greatest anomalies in scientific history. As the only vertebrates to have clear blood, the Antarctic Icefish have abandoned a way of life that once supported its ancestors in the past, and one that supports animals in the present. The event that triggered this anomaly was the deletion of the β-globin subunit of the hemoglobin gene, rendering the gene to be completely obsolete. In addition to the loss of the hemoglobin gene, the myoglobin gene was also lost in all of the icefish species. Because Icefish could no longer transport oxygen through their blood, they lost their scales and adapted by diffusing oxygen into their blood plasma through their enlarged gills instead. The Icefish also have antifreeze proteins that bind to ice crystals to hinder their growth. It is in this manner that they are able to survive in the frigid waters of the Antarctic.

Introduction

Ten million years ago, Antarctica broke off from South America, causing a new current to circle Antarctica, thereby dropping ocean temperatures to -1.9 degrees Celsius. Over the following years, many of the fish migrated or died, but some fish persevered. Through many years of evolution, genetic mutations, and natural selection, the Icefish eventually grew to be the first vertebrates to lack hemoglobin. In 1927, a Norwegian zoologist named Ditlef Rustad first discovered the evolutionary anomalies.[1] He fileted the fish to discover that all of its organs were white, and its blood was transparent.

The Crocodile Icefish, or the Channichthyidae, is a species of fish that inhabit the Southern Oceans of Antarctica. Hundreds of years ago, the Icefish's hemoglobin gene underwent a mutation, which rendered the gene to be completely useless, while simultaneously giving the fish its notoriously clear blood. Because of the freezing temperatures of the Antarctic Oceans, however, this mutation ultimately benefited the Icefish, as animals with hemoglobin would have died from lack of blood flow in the icy temperatures of the Antarctic Oceans. Although hemoglobin was an essential part of transporting oxygen throughout their bodies, the Icefish found another way to absorb oxygen: they lost their scales and absorbed oxygen through their skin.

Now, another question was raised: how did the Icefish, under such frigid conditions, have blood coursing through its veins? In 1960, scientist Art Devries discovered that Icefish were able to create an antifreeze protein that would bind to ice crystals and prevent them from growing.[1] This development was the effect of another mutation in the animal's DNA when an ancestral gene was accidentally duplicated. This duplication produced a copy of the gene that gave birth to this revolutionary protein.

The Channichthyidae proves that even if one species has followed a way of life for millions of years, it can still change and adapt as the world advances. The potential of genetic mutation and variation is unfathomable, and the Icefish is just one example of many to come.

Discussion

Hemoglobin and Myoglobin Gene Mutation

Antarctic Icefishes are known to be a scientific anomaly because they lack hemoglobin and erythrocytes - both of which are actively present and critical to the function of many animals. Hemoglobin (Hgb) is the metallo-protein that bonds with oxygen in almost all of the vertebrates, and some invertebrates. Usually, hemoglobin consists of four protein subunits, including two units of α-globin (HBA) and β-globin (HBB), which all help transport oxygen to different parts of the body.[2]

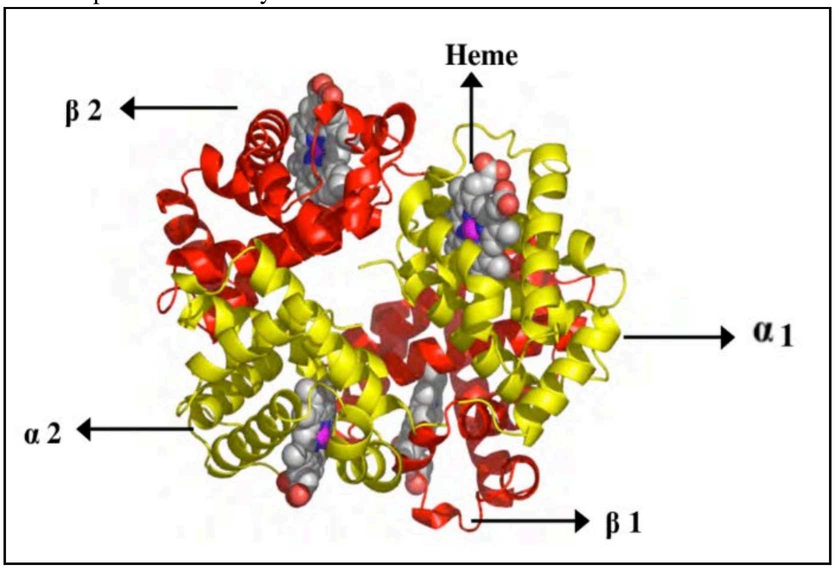

Figure 1. Subunits of hemoglobin[3]

In a study published by the *National Library of Medicine*, scientists discovered that fifteen of sixteen icefishes were found to have lost the β-globin gene, but still retain some of the α-globin pseudogene.[4] The retained α-globin gene is identical to the gene's DNA sequences present in red-blooded fish, containing all of exon 3, part of intron 2, and the untranslated region of the 3' strand of DNA. It is believed that the inability to express hemoglobin originated from one event, where all of the globin genes were deleted, excluding the 3' end of the α-globin gene.[5]

In addition to the loss of hemoglobin, myoglobin (Mb) expression was also lost in the Icefish genome. However, it isn't completely gone. In the family of Antarctic Icefishes, or the Channichthyidae, only 5 species have Mb present in heart ventricles, while Mb is absent from the heart auricle and oxidative skeletal muscle in all of the species. Scientists believe that the loss of Mb expression has occurred at least three times in the past.[6]

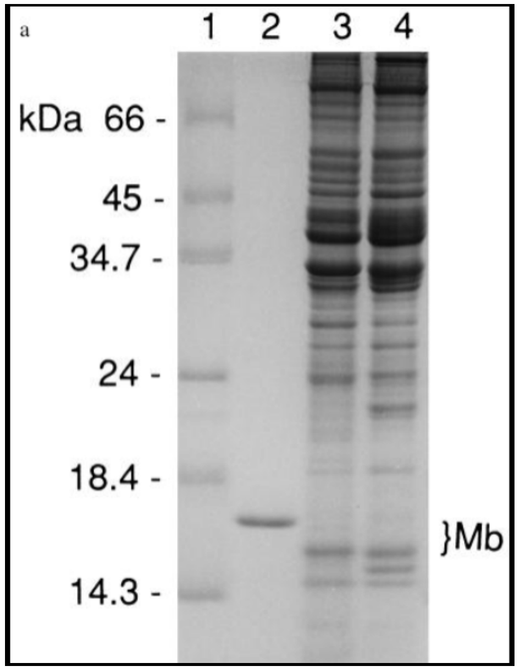

Figure 2. The presence of myoglobin in the heart ventricles of Antarctic Icefishes (Lanes: 1, protein weight standards; 2, human heart myoglobin standard; 3, C. aceratus; 4, C. rastrospinosus)[7]

While "Quanta Magazine" labels this mutation to be a "lucky accident"[8], the "Nature and Ecology Journal" believes it to be a result of the extreme conditions that the Icefish were subjected to. To prove that the strong environmental changes affected the genomes, the scientists at the "Nature and Ecology Journal" conducted an experiment where they sequenced the genome of a female Antarctic blackfin Icefish using PacBio Sequel instruments, which yielded 90x genome coverage and a 13-kilobase read length. The genome size was about 1.1 giga base pairs, which was evaluated to be 89.9% complete and 3.6% fragmented.[9] Small-RNA transcriptomics from five tissues allowed for the annotation of microRNAs, or single-stranded non-coding RNA molecules, which identified 290 miRNA genes and 334 unique miRNAs. The scientists found that the icefish genome has 50.4% repetitive sequences, and most were transposable elements, which are elements in a DNA sequence that can change position within a genome and can create or reverse mutations.[9] By calculating the relative age of the transposable elements, the experiment showed a recent burst of DNA transposons and long and short interspersed elements. This confirms the hypothesis that strong environmental changes can lead to massive changes in transposable elements.

From those mutations, the Icefishes' blood is now made up of various proteins and macromolecules that help keep the blood functioning under the frigid waters, but no hemoglobin. Many other animals like the Icefish had to adapt to their environment and they eventually evolved to have different variants of hemoglobin. Animals like octopuses and crabs, for example, have hemocyanin, which is a variant of hemoglobin that is advantageous when used in high oxygen environments.[8] The Icefish, however, lives in such an extreme environment, where any existing variant of hemoglobin would not be able to function properly.

Consequently, the Icefish blood can only carry less than 10% of the oxygen that its red-blooded relatives can carry. This is because the clear blood can only transport oxygen in a physical solution, so it is limited in how much it can carry. To compensate, the Icefish have large hearts with increased stroke volume and enhanced vascular systems.[10]

The Antifreeze Protein

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To survive in the icy waters in Antarctica, the Channichthyidae have an antifreeze glycoproteins (AFGP) that lowers the body's freezing point, and they also bind to ice crystals, inhibiting their growth through an absorption-inhibition process that is still being researched today.[11] Usually the AFGP gene is made up of a simple glycotripeptide monomeric repeat, and the gene encodes a large polyprotein precursor that has as many as 46 AFGP molecules linked together by tripeptide spacers (Leu/Phe-ile/Asn-Phe).[12] The AFGP genes are recently found to evolve from a pancreatic trypsinogen gene, which is the substance that starts the process to break down proteins in the pancreas. Scientists believe that segments of the trypsinogen gene, in addition to the *de novo* amplification of a Thr-Ala-Ala coding, form a new coding region for the tripeptide backbone of the AFGPs.[12]

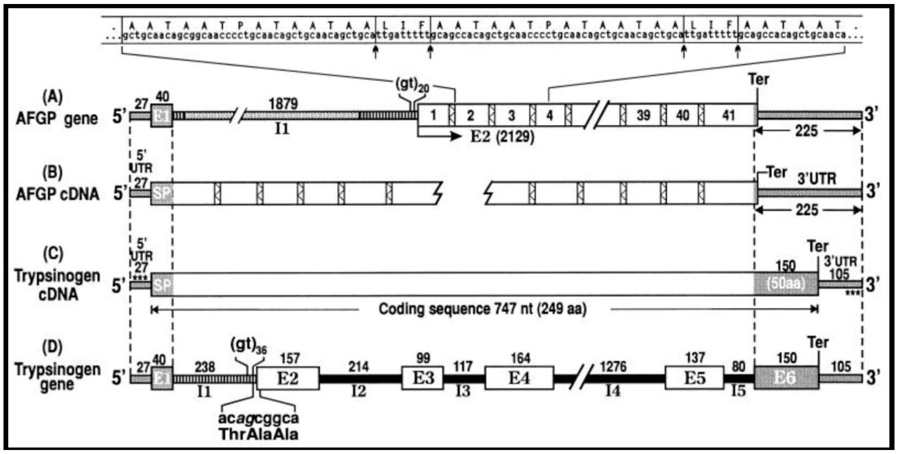

Figure 3. Structures of AFGP and Trypsinogen genes and cDNAs from Antarctic Icefish [13]

The antifreeze proteins are applicable to many different uses, as they are almost three-hundred times more effective to prevent freezing compared to chemical antifreezes in the market today. The fish antifreeze can be studied to prevent freezing of food or freezing injuries. They can be used in the cryopreservation of foods or organs. The observation of AFGPs can provide more perspectives on how biomedical molecules affect bio-crystals like kidney stones.

Conclusion

The Antarctic Icefish are an anomaly in the animal kingdom, as they are peculiar for having clear blood, large circulatory systems, antifreeze proteins, but they also represent a new potential breakthrough in modern-day medicine. Because this family of fish have the ability to overcome harsh temperatures in their environment, they could be the potential cure for anemia, which is the condition where there is a lack of red blood cells to carry oxygen. With a grand total of zero red blood cells, while still transporting oxygen, the Icefish pose themselves as research models to many scientists seeking to find the answers to this illness.

Additionally, the Icefish, over time, have developed floppy bones that are less mineralized compared to its ancestors. They allow for buoyancy, which helps the fish rise in the water and catch their prey. By studying this aspect of the animal, scientists could learn more about osteoporosis and how humans lose bone density with age.

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References

1. biointeractive. “The Icefish Has Clear Blood - HHMI Biointeractive Video.” *YouTube*, YouTube, 26 Aug. 2014, https://www.youtube.com/watch?v=WpIe8_pUSu4.
2. PDB101: Molecule of the Month: Hemoglobin. *RCSB*, https://pdb101.rcsb.org/motm/41.
3. *Figure 1.1: Tetrameric Structure of Human Hemoglobin. the Alpha Chains...* https://www.researchgate.net/figure/Tetrameric-structure-of-human-hemoglobin-The-alpha-chains-a1-a2-are-shown-in-yellow_fig1_323322520.
4. HW;, Near TJ;Parker SK;Detrich. “A Genomic Fossil Reveals Key Steps in Hemoglobin Loss by the Antarctic Icefishes.” *Molecular Biology and Evolution*, U.S. National Library of Medicine, https://pubmed.ncbi.nlm.nih.gov/16870682/.
5. Zhao Y;Ratnayake-Lecamwasam M;Parker SK;Cocca E;Camardella L;di Prisco G;Detrich HW; “The Major Adult Alpha-Globin Gene of Antarctic Teleosts and Its Remnants in the Hemoglobinless Icefishes. Calibration of the Mutational Clock for Nuclear Genes.” *The Journal of Biological Chemistry*, U.S. National Library of Medicine, https://pubmed.ncbi.nlm.nih.gov/9614073/.
6. Sidell BD;Vayda ME;Small DJ;Moylan TJ;Londraville RL;Yuan ML;Rodnick KJ;Eppley ZA;Costello L; “Variable Expression of Myoglobin among the Hemoglobinless Antarctic Icefishes.” *Proceedings of the National Academy of Sciences of the United States of America*, U.S. National Library of Medicine, https://pubmed.ncbi.nlm.nih.gov/9096409/.
7. Sidell BD;Vayda ME;Small DJ;Moylan TJ;Londraville RL;Yuan ML;Rodnick KJ;Eppley ZA;Costello L; “Variable Expression of Myoglobin among the Hemoglobinless Antarctic Icefishes.” *Proceedings of the National Academy of Sciences of the United States of America*, U.S. National Library of Medicine, https://pubmed.ncbi.nlm.nih.gov/9096409/#&gid=article-figures&pid=figure-1-uid-0.
8. Rennie, John, and substantive Quanta Magazine moderates comments to facilitate an informed. “Icefish Study Adds Another Color to the Story of Blood.” *Quanta Magazine*, 27 June 2019, https://www.quantamagazine.org/icefish-study-adds-another-color-to-the-story-of-blood-20190422/.
9. Kim, Bo-Mi, et al. “Antarctic Blackfin Icefish Genome Reveals Adaptations to Extreme Environments.” *Nature News*, Nature Publishing Group, 25 Feb. 2019, https://www.nature.com/articles/s41559-019-0812-7.
10. Jabr, Ferris. “How the Antarctic Icefish Lost Its Red Blood Cells but Survived Anyway.” *Scientific American Blog Network*, Scientific American, 3 Aug. 2012, https://blogs.scientificamerican.com/brainwaves/how-the-antarctic-icefish-lost-its-red-blood-cells-but-survived-anyway/.
11. *Fish Antifreeze Proteins*, https://www.nsf.gov/pubs/1996/nstc96rp/sb3.htm#:~:text=To%20survive%2C%20Antarctic%20fishes%20have,proteins%20were%20discovered%20by%20Dr.
12. Chen, L, et al. “Convergent Evolution of Antifreeze Glycoproteins in Antarctic Notothenioid Fish and Arctic Cod.” *Proceedings of the National Academy of Sciences of the United States of America*, The National Academy of Sciences of the USA, 15 Apr. 1997, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC20524/#B12.
13. CH;, Chen L;DeVries AL;Cheng. “Evolution of Antifreeze Glycoprotein Gene from a Trypsinogen Gene in Antarctic Notothenioid Fish.” *Proceedings of the National Academy of Sciences of the United States of America*, U.S. National Library of Medicine, https://pubmed.ncbi.nlm.nih.gov/9108060/#&gid=article-figures&pid=figure-1-uid-0.