

CHANGING THE COURSE OF HISTORY, ONE GENE COPY AT A TIME

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Evolution is important for the survival of a species because it allows the species to adapt to environmental changes. This can occur through mutations, natural selection, or other mechanisms such as gene duplication. Gene duplication allows the original version of a gene to be maintained in the genome, while a copy of that gene can undergo genetic changes without affecting the function of the original gene. One group of genes that have evolved through gene duplication are the primate alpha-amylase genes. Alpha-amylases are crucial enzymes for starch digestion because they allow for the cleavage of complex sugars, such as starch, into simpler sugars that can be more easily absorbed through nutrient uptake. During human evolution, gene duplication gave rise to three types of alpha-amylases: salivary amylase (AMY1) and pancreatic amylases (AMY2A and AMY2B). The pancreatic amylases are expressed in the pancreas and travel to the small intestine, while the salivary amylase, expressed in the salivary glands, travels to the oral cavity and then to the stomach. Since these amylases are found in different environments, they evolved to function optimally in differing pH conditions. Previous studies in our lab have shown that AMY1 (salivary) has higher enzymatic activity than AMY2A (pancreatic) in acidic environments (pH 3-6), but low activity in neutral environments. Although these observations have been made, it is unclear how each amylase changed its function to adapt to different pH environments. From this, I hypothesize that after gene duplication AMY1 and AMY2A evolved independently to maximize their activities in acidic and neutral pHs, respectively. To test this hypothesis, I resurrect the enzyme ancestral to AMY1 and AMY2A and will compare its properties with those of present-time enzymes (AMY1 and AMY2A). For this, I use the gene sequence of the ancestral gene determined by statistical inference. This sequence is chemically synthesized and inserted into the yeast (*Pichia pastoris*) genome. Then, its proteins are expressed, purified, and tested for their activities in acidic and neutral pHs using an enzymatic assay. With this data, we will elucidate the evolutionary history of the human alpha-amylase genes that contributed to our adaptation to a higher starch diet.