Evolution of an enzyme in a multigene family: the enigmatic role of Amyrel, a paralog of alpha-amylases in flies

Jean-Luc Da Lage¹, Magalie Bonneau¹, Eliza Gondim de Sá¹, Isabelle Robineau¹ and Georges Feller²
¹: EGCE, CNRS and University Paris-Saclay; F- 91198 Gif sur Yvette; ²: Laboratory of Biochemistry, Center for Protein engineering, University of Liège B-4000

Amylases are almost ubiquitous in the living world. They perform the cleavage of glycosidic bonds in starch and glycogen. In many organisms, they are encoded by multiple gene copies that are more or less divergent. This multigene structure permits increased enzyme production or adaptation to various starchy foods or to various conditions (tissue-specificity, pH, inhibitors).

In the Muscomorpha (true flies), a paralog named Amyrel (Amylase-related) has been conserved for more than 100 MY. Yet, its function is still elusive.

Amyrel diverged from the classical Amy gene by 40% in amino acids. Amyrel sequences are well clustered in a tree of dipteran amylases. The protein has particular features: loss of a GHGA motif in an important loop; additional disulfide bridge [1].

To try to understand the biological function of Amyrel, we investigated direct and indirect clues, e.g. if the gene is properly regulated, if the encoded protein has enzymatic abilities, and if Amyrel mutants have any visible phenotype that could suggest a physiological role. And thus we have to answer the question:

To what extent is Amyrel similar or different from a classical amylase?

Amyrel has a weak amylolytic activity compared to Amy (ca. 30x less). But it has two additional enzymatic activities: glucosidase (releases glucose from maltose) and glycosyltransferase (cuts pastes oligosaccharides), and is able to hydrolyze the maltolinoze, contrary to Amy. These activities are antagonistic and simultaneous!

Analysis of an Amyrel-null mutant: a null mutant was obtained using CRISPR/Cas9, and was compared to the wildtype (wt) for some life history traits on a standard diet: no difference was found for pre-emergence mortality, average weight or lifespan. Only the development time to adult emergence was found slightly shorter in the mutant.

A competition experiment is going on between the null and the wt allele (in the same genetic background) on three different diets: after 25 generations, no advantage appears for either the mutant or the wt.

Conclusion: Amyrel is an active enzyme with very original enzymological properties. The gene undergoes strict regulation, most similar to Amy, but its inactivation seems not detrimental in lab conditions. However, wild life is different from lab life, and the conservation of the gene for such a long time suggests a specific function. The quest must go on!

References