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

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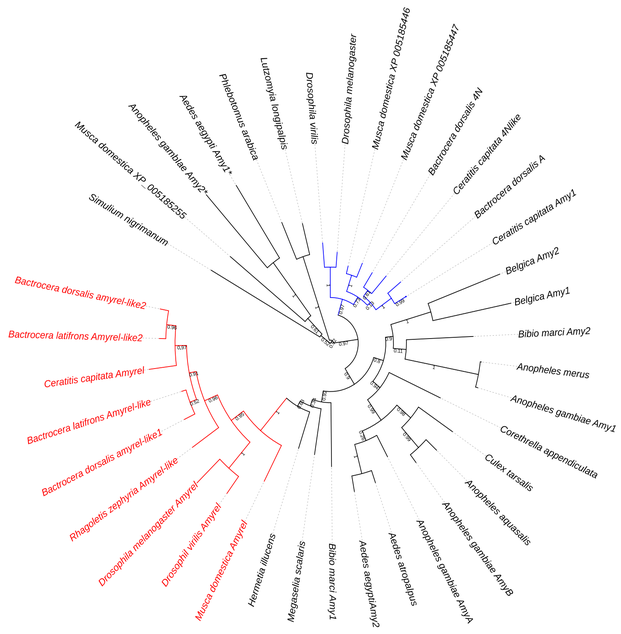
Context

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]...

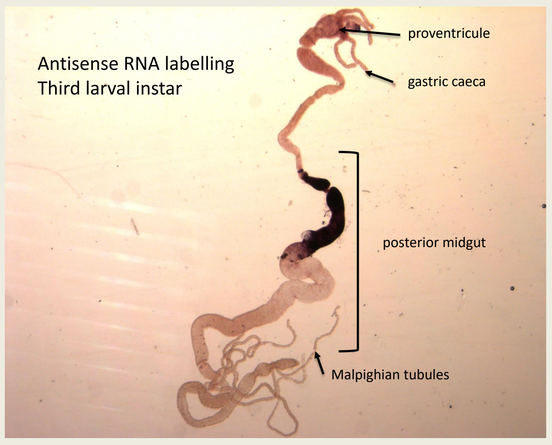


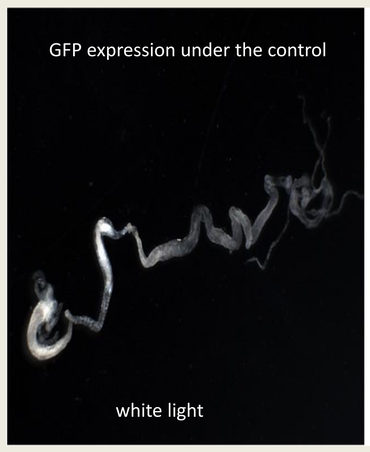
ML tree of dipteran amylase protein sequences. The tree was built using phylogeny.fr [2] and drawn using iTOL [3]. Amyrels are in red, Amy counterparts are in blue.

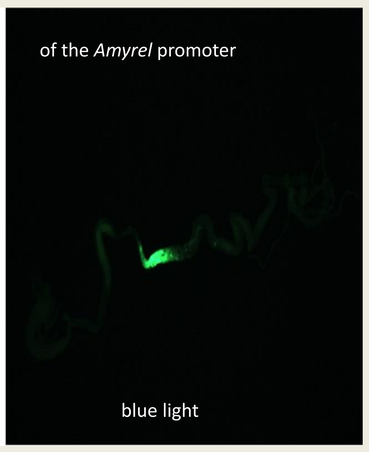
To try to understand the biological function of Amyrel, we investigated direct and indirect clues, e.g. if the gene is properly regulated, if the encoded protein has enzymatic abilities, and if Amyrel mutants have any visible phenotype that could suggest a physiological role. And thus we have to answer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

The study

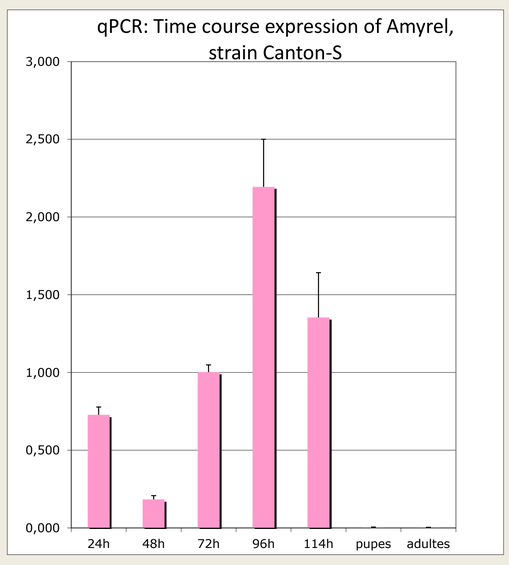
To what extent is Amyrel similar or different from a classical amylase ?

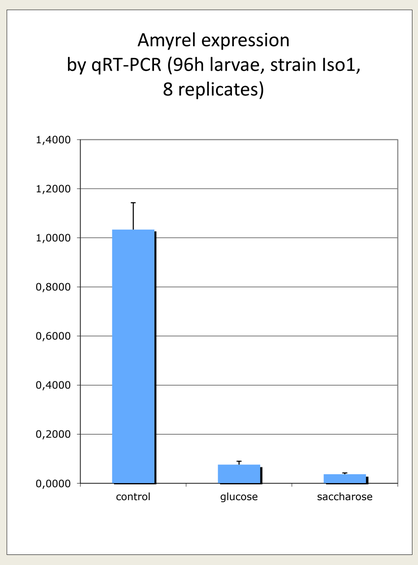






Expression takes place in the midgut, like the classical amylase, but in a limited part.

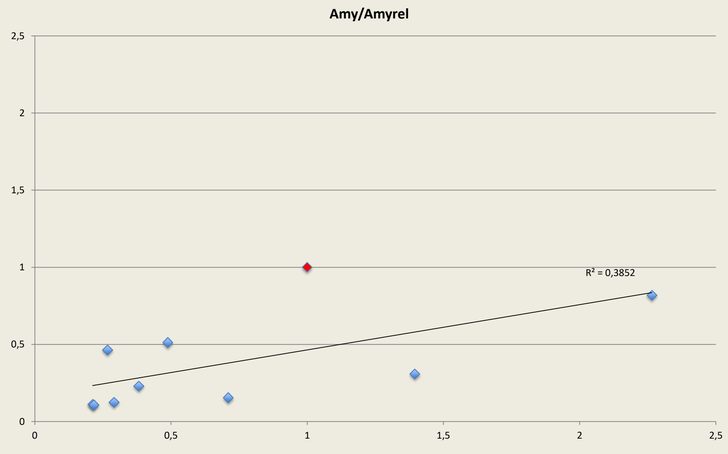




Amyrel is not expressed in adults !

Amyrel expression is downregulated by sugars, like *Amy* [4].

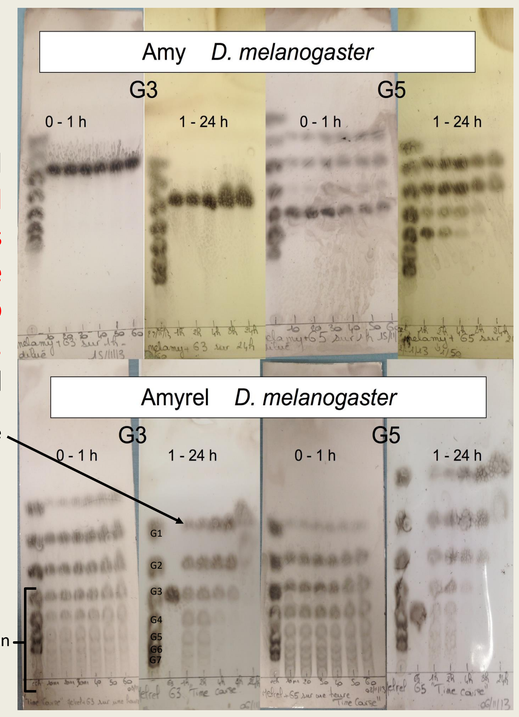
Regulation



Expressions of *Amy* and *Amyrel* were compared in each of ten *D. melanogaster* strains from various origins. There is no significant correlation in their relative expression, i. e. a strain may have higher *Amy* and lower *Amyrel* expression. The reference strain was Canton-S (red dot).

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 maltotriose, contrary to Amy. These activities are antagonistic and simultaneous !

Enzymology

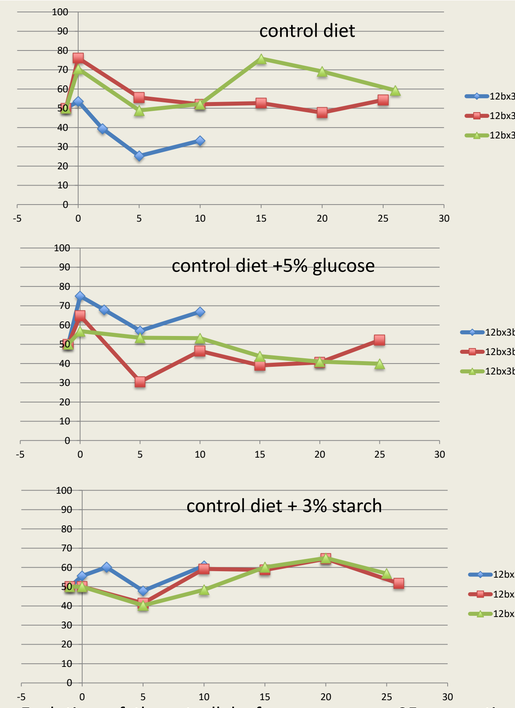


Thin layer chromatographies of digestions of maltotriose (G3) and maltopentaose (G5) at 37°C by Amy (upper panel) and Amyrel (lower panel). Both enzymes were produced *in vitro* in the yeast *Pichia pastoris*. Glucosidase activity is evidenced by appearance of glucose; transglycosylation yields oligosaccharides of various sizes.

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.

Fitness



Evolution of the wt allele frequency on a 25 generation period. Three replicates were perfomed (3 colors) for each of the three diets. Replicate 1 was stopped after 10 generations. 30 females wt/wt and 30 females null/null were the founders (50/50). A similar experiment is performed with another independent null mutant.

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
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[4]- Benkel, B. F. & Hickey, D. A. *Genetics* **114**, 137-144 (1986)

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