Introduction

Isozymes were first described by R. L. Hunter and Clement Markert (1957) who defined them as *different variants of the same enzyme having identical functions and present in the same individual*.[1] This definition encompasses (1) enzyme variants that are the product of different genes and thus represent different loci (described as *isozymes*) and (2) enzymes that are the product of different alleles of the same gene (described as *allozymes*).[2]

Isozymes are usually the result of gene duplication, but can also arise from polyploidisation or nucleic acid hybridization. Over evolutionary time, if the function of the new variant remains *identical* to the original, then it is likely that one or the other will be lost as mutations accumulate, resulting in a pseudogene. However, if the mutations do not immediately prevent the enzyme from functioning, but instead modify either its function, or its pattern of expression, then the two variants may both be favoured by natural selection and become specialised to different functions.[3] For example, they may be expressed at different stages of development or in different tissues.[4]

Allozymes may result from point mutations or from insertion-deletion (indel) events that affect the coding sequence of the gene. As with any other new mutations, there are three things that may happen to a new allozyme:

- It is most likely that the new allele will be non-functional—in which case it will probably result in low fitness and be removed from the population by natural selection.[5]
- Alternatively, if the amino acid residue that is changed is in a relatively unimportant part of the enzyme (e.g., a long way from the active site), then the mutation may be selectively neutral and subject to genetic drift.[6]
- In rare cases, the mutation may result in an enzyme that is more efficient, or one that can catalyse a slightly different chemical reaction, in which case the mutation may cause an increase in fitness, and be favoured by natural selection.[6]

Distinguishing isozymes

Isozymes (and allozymes) are variants of the same enzyme. Unless they are identical in their biochemical properties, for example their substrates and enzyme kinetics, they may be distinguished by a biochemical assay. However, such differences are usually subtle, particularly between *allozymes* which are often neutral variants. This subtlety is to be expected, because two enzymes that differ significantly in their function are unlikely to have been identified as *isozymes*.

While isozymes may be almost identical in function, they may differ in other ways. In particular, amino acid substitutions that change the electric charge of the enzyme are simple to identify by gel electrophoresis, and this forms the basis for the use of isozymes as molecular markers. To identify isozymes, a crude protein extract is made by grinding animal or plant tissue with an extraction buffer, and the components of extract are separated according to their charge by gel electrophoresis. Historically, this has usually been done using gels made from potato starch, but acrylamide gels provide better resolution.