Glutamate synthase: An archaeal horizontal gene transfer?

The availability of several new genome sequences from a variety of species of different evolutionary levels has brought interesting information about the possible existence of horizontal gene transfer between taxons. An interesting example of such a transfer may well be the case of glutamate synthase (GOGAT) which is a key enzyme in ammonia assimilation in bacteria, algae and plants. It catalyzes the reductive transamidation of amido nitrogen from glutamine to 2-oxoglutarate to form two molecules of glutamate (Temple *et al* 1998)

Glutamate synthases differ according to their molecular weights, subunit compositions, immunological characteristics, and electron donor specificities. They contain iron-sulphur cluster(s), FAD and/or FMN as prosthetic groups (Knaff *et al* 1991). Several glutamate synthases from a variety of taxonomic groups such as eubacteria, cyanobacteria, algae, yeast and plants have been cloned and sequenced (Nalbantoglu *et al* 1994). A cyanobacterial (Navarro *et al* 2000) and a bacterial glutamate synthase have been expressed in *Escherichia coli* (Vanoni *et al* 1996) and site-directed mutagenesis experiments performed to determine the NADPH binding region which is the electron donor of some glutamate synthases (Morandi *et al* 2000).

Bacterial glutamate synthases consist of two non-identical subunits and use NADPH as an electron donor (Pelanda *et al* 1993). The large subunits of bacterial glutamate synthases are highly homologous to the monomeric ferredoxin-dependent glutamate synthases of 160–180 kDa (Nalbantoglu *et al* 1994; Suzuki and Rothstein 1997). Some plants such as alfalfa also have a single subunit NADH-dependent glutamate synthase of a relatively bigger size, approximately 235 kDa (Gregerson *et al* 1996). The N-terminal region of this enzyme shows homology to the large subunit of bacterial GOGATs while the C-terminal region is homologous to the small subunit of the bacterial enzymes. The analysis of enzyme activity of *Azospirillum brasiliense* GOGAT showed that the large subunit has the *PurF*-type of glutamine amido transferase site (Pelanda *et al* 1993) while the NADPH reduction site was shown to be located in the small subunit (Morandi *et al* 2000).

The elucidation of several archaeal genomes in the recent years brought new insights to the gene organization of glutamate synthases in archaea (Bult *et al* 1996; Kawarabayasi *et al* 1998). It has been observed that the methanogenic archaeon *Methanococcus jannaschii* genome has a putative GOGAT of one third of the length of the large bacterial subunits: This is a 1·5 kb open reading frame which does not have a small subunit coding gene upstream or downstream. A small subunit-like region has not been found in any other part of the 1·7 megabase pair genome. This putative GOGAT shows high homology to the other glutamate synthases in its C-terminal region including the strictly conserved cysteines which are the ligands for the Fe-S cluster.

The ferredoxin-dependent GOGATs are functional enzymes without a small subunit although no bacterial GOGAT has been shown to be active as a single subunit. As archaea have been shown to have a more recent common ancestor with eukaryotes than eubacteria in evolution according to 16S rRNA data, a single subunit archaeal GOGAT may be considered a possibility which requires further experimental analysis for conclusion.

Interestingly, two other archaeal species, *Pyrococcus horikoshii* and *Pyrococcus abysii* have two open reading frames which are distantly located on their genomes and they show high homology to small subunit-like GOGAT region and none of the four open reading frames has a large subunit-like region either upstream or downstream (Dincturk and Knaff 2000). The presence of two different small subunit-like regions in the genome with high homology to one another suggests a possible gene duplication. Another single small subunit-like GOGAT gene has been cloned from another *Pyrococcus* species and expressed in *E. coli*. It has been claimed that the purified recombinant protein is a functional enzyme as a tetramer (Jongsareejit *et al* 1997). This is the only report for GOGAT activity of the small subunit without the presence of a big subunit. This is rather interesting as this subunit has been attributed to play a role in NADPH-binding but not glutamine amidotransferase activity.

Could it be that the two subunits were two separate enzymes earlier in evolution and were later joined via horizontal gene transfer to provide an NADH-binding region to some higher plant GOGATs? Or as they are two separate subunit enzyme in bacteria, were they separated in archaea when another electron donor became available or more abundant? Which of the electron donors could

be earlier in evolution? Can a tetramer structure of the small subunit bring the essential amino acids in such a three-dimensional structure which allows glutamine amidotransferase activity which is shown to be located at the large subunit? Which electron donors do the archaeal enzymes use?

In short, glutamate synthase is an excellent candidate to be a model enzyme to study horizontal gene transfer in archaea.

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