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"Challenging problems for the understanding of chitin biosynthesis"

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Chitin is amply distributed in nature, but only in eukaryotic kingdoms. In fungi, chitin is responsible for the mechanical resistance of the cell wall. Study of chitin synthesis is important from both basic and applied points of view. The latter since it represents the ideal target for antimycotics. Additionally, the biosynthetic enzyme chitin synthase (Chs) may be subject to genetic engineering for increased production of the polysaccharide as substitute for non-degradable plastics in a number of applications. Despite of our increased knowledge on Chs, its mechanism of action remains elusive. Whether a glucosyl acceptor and a high-energy intermediate are involved in the reaction is unknown. Some data also suggest that the biosynthetic mechanism may occur in two steps. Chitin synthases are synthesized in the ER and mobilized in the cell through the normal exocytic route. Specialized microvesicles known as chitosomes, target the enzyme to the sites at the surface where the polysaccharide is synthesized. Interestingly, coding genes (*CHS*) have been more widely studied than the enzymes themselves. A general feature of fungi is to contain more than one Chs. Analysis of the aa sequence of a large number of Chs reveals that they are grouped into two divisions and five classes represented in all fungal major taxa. Multiple alignment of chitin synthases have established four kinds of hierarchical similarities: i) sequences specific to each class; ii) those common to each division; iii) sequences conserved in each one of the different kingdoms represented; and finally iv) those sequences conserved in all chitin synthases. Accordingly it appears that division of Chs groups started soon after fungi separated during evolution from the rest of the eukaryotic kingdoms. Chitin synthases have scant similarities with other proteins. Search for proteins with homology to any chitin synthase in genomic banks gives only significant positive results with other Chs. Also use of programs that look for short sequences of homology or structural motifs, such as Pfam do not recognize any common homologue in the genomic banks. Nevertheless different studies have revealed that Chs belong to the $(\beta/\alpha)_8$ -barrel fold family of proteins, and conserve the essential features of β-glycosyl transferases, *i. e* the QXXRW motif, and three aspartic or asparagine residues at the catalytic site. The other conserved sequences and amino acids identified in all Chs are QXXEY; LPG, EDRXL, and invariant aa residues that keep the form of the protein folded as alternating α/β type. Chitin synthases are highly hydrophobic proteins with several transmembrane domains. The QXRRW pentapeptide is probably located at the surface in a short hydrophilic stretch, catalogued as a flexible region, followed by hydrophobic domains. It is interesting to notice that all Chs maintain the same structural features and the same relative location of the conserved domains.

Globally the results indicate a noticeable conservation of the protein structure, independently of the changes occurring in the amino acid sequence. This feature suggest that the relative location of certain important residues, and the secondary structure of the peptide have been conserved during evolution, and are responsible for the preservation of catalytic activity in chitin synthases belonging to organisms widely separated in evolution.