Chitin Metabolism

Description

The coordination of chitin synthesis and its degradation re-quires strict control of the participating enzymes for subsequent growth and development in insects and fungi. As for insects, growth and morphology depend on the capability to remodel, in time and space, chitin-containing structures. Insects, repeatedly produce chitin synthases and chitinolytic enzymes in different tissues.Merzendorfer & Zimoch, 2003 A similar interplay be-tween synthesis and degradation occurs in cell division and sporulation of yeast and other fungi.

3.1. Chitin biosynthesis
Chitin biosynthesis follows three distinct steps. In the first step, the enzymes' catalytic domain facing the cytoplasmic site forms the polymer. In the second step, the nascent polysaccharide chain translocates throughout the membrane from where it is released into the extracellular space. The third step completes the process, and single polysaccharide spontaneously assembles to form crystalline microfibrils.

All the different chitin forms are the result of biosynthesis by glycosyltransferases : chitin synthases (UDP-N-acetyl-D-glucosamine : chitin 4-?-N-acetylglucosaminyltransferase). These Glycosyltransferases (GTs) are plasma membrane-associated. They are highly conserved enzymes present in every chitin-synthesizing organism. The CAZy database classifies chitin synthases as belonging to the GT-2 family.Henrissat, 1993 On top of chitin synthases, this family contains other inverting GTs synthases, such as cellulose synthases, and hyaluronan synthases. These enzymes utilize UDP-N-acetylglucosamine (UDP-GlcNAc) as a substrate and require divalent cations as co-factors. Addition of GlcNAc to the non-reducing end of the polymer elongates chitin polysaccharide. In this process, the nucleotide sugar donor UDP-GlcNAc is transferred to the ?-linked GlcNAc sugar in an inverting mechanism onto the non-reducing end of the growing acceptor chain.

There exists a large literature available on the synthesis of chitin. All characterized fungi have chitin synthases and most have multiple genes encoding chitin synthases. *S. cerevisiae* has three genes that encode chitin synthases.Bansal et al., 2012 The chitin synthases are large transmembrane proteins with theoretical molecular masses ranging from 160·kDa to 180·kDa. Three distinct domains termed A, B, and C constitute the enzyme. Domain A is located at the N-terminus and has limited sequence conservation among different species. In fungal class, I–III and VI CHSs, the A domains do not contain any transmembrane helices, whereas class IV + V and VII enzymes contain 2–3 transmembrane helices. The B domain comprises about 400 amino acids and hosts the catalytic center of the protein. It is highly conserved and it contains two unique motifs that are present in all types of chitin synthases including those essential for the catalytic mechanism.Breton et al., 2001 Then follows the C-domain that contains 3-7 transmembrane helices. These transmembrane domains form a channel through which the elongating chitin chain is extruded into the cell wall space, reducing end first. Despite being the essential enzymes, chitin synthases have resisted protein expression, solubilization, and crystallization for structural studies or high throughput ligand screening. As a result, the detailed

understanding of the mode of action of the chitin synthase remains to be established. The similarities that exist between the structure and function of membrane-integrated processive glycosyltransferases might help to establish firmer the understanding of chitin biosynthesis.Bi et al., 2015

In the cell wall space, chitin polysaccharide chains assemble into microfibrils, having dimensions of about 0.3 nm, through-out intermolecular interactions involving hydrogen bonding and van der Waals interactions. Within these microfibrils, there exist significant differences between the relative arrangements of the chitin chains, leading to two distinct polymorphic types. These crystalline types are referred to as ?, ?, chitin. In the ? chitin, the polymer chains are arranged in an antiparallel fashion, whereas a parallel orientation of the chains forms ? chitin crystals. These two forms of chitin vary in packing and polarities of adjacent chains in the succeeding sheets. The length of the chitin microfibrils can be as long as 0.5 µm. Up to 10 or more of these microfibrils further organize in the form of bundles. Such type of architecture provides both thermodynamic stability and prevents accessibility to chemicals.

Many investigations concern the fungal kingdom, where multiple genes encoding chitin synthases have been identified. Such a wide diversity suggests that different chitin synthases might be used for chitin production to varying stages of the fungal life cycles such as in septum formation, hyphal growth, and development, or particular cell types and specific species.Horiuchi, 2009 Such an occurrence is far from being a surprise when one considers the different life histories, developmental processes, and ecological niches that characterize the more than, 1.5 million different species that constitute the fungal kingdom. Interestingly, some reports mention that chitin is not required for all fungal cell walls and that chitin can be a cell-type-specific cell wall.Lui et al., 2017 Some of the results established for fungi systems seem to be valid for the insect chitin synthases as well. In contrast to fungi, molecular analysis of Chitin Synthase genes has so far revealed a limited number of gene copies of nematode and insect chitin synthase genes (CHS).

3.2. Chitin degradation

In chitin-producing organisms, chitinolytic enzymes are essential for maintaining normal life cycle functions such as morphogenesis of arthropods or cell division and sporulation of yeast and other fungi. Since chitin is hard to break due to its physicochemical properties, its degradation usually requires the action of more than one enzyme type. The degrading enzymes include the following chitinases poly[1,4-(N-acetyl-?-D-glucosaminide)] glycanohydrolase, ?-N-acetylglucosaminidases (?-N-acetyl-?-D-hexosaminidase N-acetyl hexosaminohydrolase. As for lytic polysaccharide monooxygenases, N-acetylglucosaminidases different carbohydrate-binding modules enable tight binding to insoluble substrates. All of them catalyze the hydrolysis of ?-(1-4)-glycosidic bonds of chitin polymers and oligomers. Endo-splitting chitinases produce chitooligomers that are subsequently converted to monomers by exo-splitting ?-N-acetylglucosaminidases. Chitinases belong to the glycosyl hydrolase 18 familiesLombard et al., 2013, which is comprised of various proteins found in a wide range of organisms, including plants, bacteria, fungi, insects, protozoa, and mammals

3.2.1. ***Insects***. Insect growth and development is strictly dependent on the capability to remodel chitinous structures. Therefore, insects consistently synthesize and degrade chitin in a highly controlled manner. During each molt cycle, a new cuticle is deposited simultaneously with the degradation of the inner part of the chitinous procuticle of the overlying old exoskeleton by molting fluid enzymes including epidermal chitinases. Degradation of cuticles by chitinolytic enzymes certain-ly needs the assistance of molting ?uid proteases to degrade proteinaceous components. The mechanism of catalysis seems to

be similar to that postulated for the cellulase complex and other multi-enzyme systems hydrolyzing polysaccharide assemblies.

Insect chitinases are endo-splitting enzymes that retain the anomeric ?-(1,4) configuration of the cleavage products. They belong to family 18 of the glycoside hydrolase superfamily (GH18). However, some of them have lost their catalytic activity but retained the chitin-binding activity and possess growth factor activity. In all sequenced insect genomes, multiple genes encode chitinases. They are differentially expressed during development and in various insect tissues. Some of them have non-redundant functions and are essential for growth and development. A particular property is their multi-domain architecture, which comprises varying numbers of catalytic and chitin-binding domains that are connected by glycosylated serine/threonine linker regions. Based on sequence similarities and organization, they have been classified into eight different groups.

3.2.2. ***Fungi****.* Fungal cell walls confer mechanic stability during cell division and polar growth. Chitinases play a house-keeping function in plasticizing the cell wall or can act more specifically during cell separation, nutritional chitin acquisition, or competitive interaction with other fungi. In the case of phytopathogenic fungi, the cell wall is the first constituent that establishes intimate contact with the host plant. Depending on the species and lifestyle of fungi, there is a considerable variation in the number of encoded chitinases and their function.

Category

1. News