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Go fly a chitin: the mystery of chitin and chitinases in vertebrate tissues

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1. ABSTRACT

A controversy arose decades ago whether the DG42 gene product expressed during frog embryogenesis synthesized hyaluronan or chitin. Both sets of investigators were correct. It is now possible to understand how prescient those findings were. Synthesis of a seven to nine chitin sugar chain fragment is required before hyaluronan synthesis begins. Thus, DG42 indeed synthesizes both hyaluronan and chitin. Hyaluronan turns over rapidly in vertebrate tissues, but chitin oligomers are difficult to degrade. They accumulate and can cause pathology. Chitin is a simple beta-linked repeating sugar homopolymer found prominently in the building block structures of fungi, molluscs, arthropods, and other forms of invertebrate life. It is a highly resistant insoluble material requiring chitin synthases for production and chitinases for degradation. Mysteriously, chitins and chitinases also occur in vertebrate tissues, while it had previously been assumed that no chitins were contained therein. That assumption is now challenged based on recent biochemical evidence. Chitin does accumulate in many tissues, but may be particularly toxic to neurons. Its accumulation in the brain may account for the cognitive decline found in patients with Alzheimer's disease. The DG42 observations together with the participation of chitins and chitinases in several human diseases, among which in addition to Alzheimer's disease include Gaucher's disease, asthma, and aspects of abnormal immune recognition justify a reexamination of these topics. The purpose of this review is to summarize data in order to place chitins and their attendant enzymes in a rational framework in an attempt to create a cohesive story.

2. INTRODUCTION

In the last decade of the 20th Century, a controversy arose as to whether an early gene product in frog development synthesized chitin or HA (hyaluronan). The product of the DG42 gene expressed during gastrulation in developing frog embryos was documented to synthesize chitin (1,2). This was surprising because it had been assumed up to that time that chitin did not occur in vertebrates. Chitin was the exclusive domain of fungi, molluscs, arthropods such as crustaceans, insects, and spiders, as well as other forms of invertebrates. The DG42 gene is expressed during a short interval during

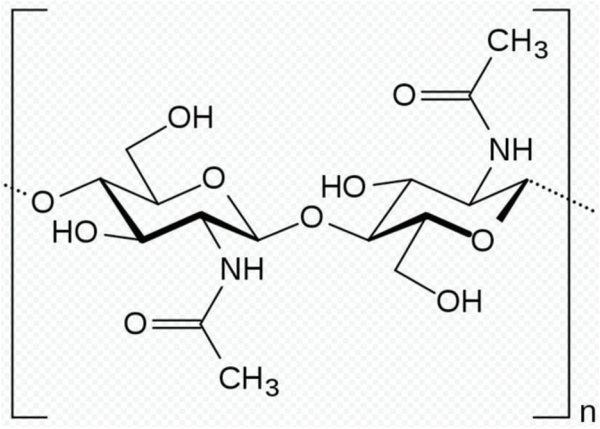

Figure 1. Structure of chitin molecule, demonstrating two N-acetyl glucosamine units that repeat forming long chains in a strictly β-1, 4 linkage.

embryogenesis of the African clawed frog *Xenopus laevis.* It is detected at mid-blastula, peaks at gastrula, and decays by the end of neurulation, the products moving as a wave through the embryo (3).

However, other equally valid studies at about the same time documented that the DG42 gene product expressed HA (4). It turned out subsequently that both are correct (5). The basis of this apparent paradox has remained an enigma for over two decades. Subsequent work has shown that these seemingly contradictory observations fit into a reasonable biological story. That story is provided here, plus additional items that fit into an intriguing and ever-expanding glycobiology landscape.

3. CHITIN

Chitin is a long chain insoluble carbohydrate homopolymer consisting of repeating units of N-acetyl glucosamine in beta-1, 4 linkages (Figure 1). Chitin is the second most abundant molecule in the biosphere, second only to cellulose. It occurs as a key structural molecule in the exoskeleton of invertebrates and cell walls of fungi. Some corals (6), and as unlikely an organism as sea anemones (7) also contain chitin. More than 10 gigatons (10^{13} kg) of chitin are synthesized and degraded in the biosphere each year (8).

The beta-linkage of chitin is similar to that in cellulose. However, one hydroxyl group of each chitin monomer is replaced with an acetyl amine group. This gives increased hydrogen bonding between adjacent polymers and provides vastly increased strength (9). The biomedical application of chitin in a variety of fields including food technology, material science, microbiology, agriculture, wastewater treatment, drug delivery systems, tissue engineering, and nanotechnology have recently been reported (10,11). Chitosan, a deacetylated form of chitin, also has very broad applications, including agricultural and biomedical uses (12,13).

Chitin is also an ancient material, appearing very early in evolution, occurring in the cell walls of fungi including that of the common yeast *Saccharomyces cerevisiae* (14). It is the site of chitin synthesis in arthropods, such as crustaceans, spiders and insects. The cell wall appears to be a very ancient site of synthesis of polymers such as chitin and HA, as discussed below.

However the ER and the Golgi apparatus are also very ancient sites of complex sugar synthesis (15). This may appear to be counter-intuitive, but the trafficking of complex sugars to and from the Golgi was already in place by the time of the divergence of the major eukaryotic lineages nearly two billion years ago. The synthesis of complex sugars was a requirement for the evolution of organisms from the very beginning (16). It may be that Golgi-derived and plasma membrane synthesized polysaccharides evolved simultaneously.

Chitin is ubiquitous in the entire biological spectrum, in part due to its unique qualities. It is translucent, pliable, resilient, and an amazingly durable material. Combined with calcium carbonate, it is an extremely strong material, as exemplified by the shells of shellfish such as oysters and clams. Chitosan is an ingredient of plastics that are biodegradable and a substrate for engineering of human tissues by use of three-dimensional bioprinting (17). Chitin's flexibility and strength make it useful as a component of surgical thread. Because of its biodegradibility, presumably by chitinase enzymes, it wears away with time as the wound heals (18). Moreover, chitin has unusual properties in that it accelerates wound healing (19-21), and also has antimicrobial activity (22). Chitin is obviously a remarkable material that we are only now beginning to understand.

There has been a long-standing assumption that vertebrates do not contain chitin. However, there is now compelling evidence that chitin is produced endogenously in some vertebrates (23). The nucleotide sequence and spatial expression pattern of one of the zebra fish chitin synthase genes has been reported that will be very useful for future studies of its functions. Chitin is found in fish, amphibians and also in mammals. Thus, there may be additional modes of chitin production other than the run of seven to nine sugars that precede the onset of HA synthesis, though there is limited evidence for this. Chitin found so far in mammals is technically these short chain oligomers, which should be distinguished from the long chains of chitin found in invertebrates and in a few vertebrates. Chitin may have roles in normal vertebrate biology, and seems to be involved in several important disease processes (23,24) as discussed below.

4. HYALURONAN

All vertebrate GAGs other than HA are synthesized by way of the Golgi apparatus and are attached to core proteins. The GAGs and the core proteins together constitute proteoglycans. The HA molecule is a unique GAG in that it is produced in an entirely different manner and is not covalently bound to a proteoglycan core protein.

Hyaluronan is a carbohydrate biopolymer consisting of alternating units of beta-1, 4 linked N-acetyl glucosamine and beta-1, 3 linked glucuronic acid. Though the structures and linkages between chitin and HA are very similar, they are diametrically opposite in physical properties. HA occupies large volumes that allow water to flow freely through the molecule and has closely associated water as well. It can form gels as in the aqueous fluids of the eye, functions as a shock absorber in the synovial fluid of joints, protecting against vessel compression during fetal delivery by means of Wharton's jelly of the umbilical cord. HA is also prominent during fetal development (25), and is the major constituent of the matrix of the normal (26) and malignant stem cell niche (27).

An impressive number of additional HA functions have been described with a vast literature that is beyond the scope of this review. The roles in the stem cell niche, embryological development, cancer, tissue repair, regeneration and wound healing are to list only a few. Much of this information can be obtained from the website Hyaluronan Today:

Glycoforum: http://www.glycoforum.gr.jp/science/hyaluronan/hyaluronanE.html

Hyaluronan expression is almost exclusively in vertebrates, with the exception of a few pathogenic bacteria such as Group A and C Streptococci (28) that appear to have stolen it from their hosts. Another singular exception is found in *Pasteurella multocida* (29).

HA emerged relatively late in evolution, appearing initially perhaps with the first chordates 450 Mya, in the sea squirt, a urochordate in the phylum Chordata. Chitin is a far more ancient polymer, with evidence that it appeared early in the Precambrian era, 2500 Mya (28). The oldest preserved chitin dates to 500 Mya in a sponge, the earliest branching organism (30).

5. HYALURONAN SYNTHASES

Hyaluronan is synthesized on the inner surface of the plasma membrane of animal cells. The HA-synthases are embedded in the plasma membrane (31,32), which parallels in many ways, the chitin synthases. The HA-synthase family, HAS1, 2, and 3 synthesize the HA polymer with strict alternating addition of the two sugars. The sugar substrates are uridine diphosphate (UDP)-glucuronic acid and UDP-*N*-acetyl glucosamine added sequentially with initiation at the reducing end of the growing polymer (33). These enzymes have a high degree of sequence homology and are all membrane embedded with seven passes on the inner surface of the plasma membrane.

The HA product is extruded through the plasma membrane into the extracellular space as it is being synthesized, permitting unconstrained polymer growth. Retention within cell would barely be possible given the size of the molecule. Small amounts of HA also occur within cells (34), but it is not clear whether such HA is synthesized directly or transported back into the cell following synthesis.

The three HAS isozymes produce different size polymers and are differentially regulated (31,32) by transcriptional, translational and post-translational levels including alternative splicing, sub-cellular localization and epigenetic processes. Most such observations come from cell culture experiments. Whether this also occurs *in vivo* has not been thoroughly established. Even though the three HA synthase enzymes are located on three different chromosomes, they have 50–71% identity, suggesting that they arose as a result of gene duplication. They occur on 19q13.4., 8q24.1.2 and 16q22.1., respectively. HAS2 synthesizes a long polymer with a MW of approximately 2×10^6 Da, and appears to be the critical isozyme. Mice with *HAS2* gene deletion die at mid-gestation (35), while gene deletions of *HAS1* and *3* have no effect on fetal development.

6. CHITIN MAY BE AN EVOLUTIONARY PRECURSOR OF HYALURONAN

It is likely that chitin synthase is an ancestral precursor of the HA synthases. It is postulated that the plasma membrane location for chitin synthesis that subsequently became the site for HA synthesis (36). Chitin and HA are both monotonous polymers without any post-synthetic modifications. Hyaluronan actually resembles chitin more closely than it resembles other GAGs.

All other GAGs including chondroitin sulfate, keratan sulfate, dermatan sulfate, heparin and heparin sulfates are synthesized on the Golgi apparatus. All of these GAGs are subject to post-synthetic modifications including sulfation and epimerization and other reactions in a highly ordered sequence-specific manner. Such complex but consistent, enzyme driven, repeating patterns of GAG modifications would only be possible on a two dimensional grid such as on a Golgi platform. Such sugar sequence precision would not be possible using a linear one-dimensional mechanism as occurs in the synthesis of chitin and HA.

Simple repeating sugars such as HA and chitin are not so constrained. The GAG modifications taking place in the Golgi apparatus include the utilization of 3′-phosphoadenosine 5′-phosphosulfate (PAPs) and its synthase. These are used for biochemical transformations involving sulfation (37,16). Sulfation within the Golgi apparatus has advantages over the inner surface of the plasma membrane, which is exposed only to cytoplasmic concentrations of this very highly specialized molecule.

Of intriguing interest is the observation that a degree of cross talk exists between HA and the Golgi-derived sulfated GAG's (38). Whether such cross talk extends also to chitin and the sulfated GAG's will require investigation.

7. CHITIN SYNTHASES

Arabidopsis, a small flowering plant of the mustard family, is the first plant to have its entire genome sequenced (39). Using motifs derived from those studies, a beta-sugar synthase superfamily was proposed. From this family of inverting nucleotide-diphospho-sugar glycosyl transferases that synthesize repeating beta-glycosyl unit structures, enzymes can be identified that include cellulose synthase, chitin synthase, HA synthase, and beta-1-3-glucan synthase (40-42). It appears therefore that many beta-sugar chain synthases may share an ancestral enzyme antecedent, an ancient sugar synthase, involved in their biosynthesis.

The biosynthetic machinery specific for chitins is highly conserved. These membrane integral enzymes catalyze the chitin polymerization reaction. Three chitin synthases are involved in the budding yeast *Saccharomyces cerevisiae* each encoded by a separate gene. A vast number of arthropod chitin synthases have been studied and characterized. These are beyond the scope of the current review and have been described elsewhere (14,43,44). More recent studies document that chitin synthases are not only more broadly distributed than had been anticipated, but also have extensive diversification (45).

A number of natural products have been identified that inhibit chitin synthase activity, most of which are antifungal agents (46,47). Structural studies however have not been advanced sufficiently so that more potent synthetic chitin synthase inhibitors can be developed. These would ultimately allow for truly rational designs of inhibitors with vast potential in agriculture, aquaculture, pesticides, as anti-fungal agents, and with many other uses in industry and medicine.

Chitin synthases may exist in vertebrate tissues that account for the recently described chitin deposition (23). However, these enzymes, other than in the zebra fish, if they exist, have not yet been identified. Their eventual isolation, characterization, and sequence analyses will certainly shed interesting insights into vertebrate evolution, as well as their various functions.

The chitin synthase uses a single UDP-sugar, while the HA synthases have evolved a bifunctional alternating UDP-sugar addition procedure. A structural mechanism for the evolution of such a bifunctional glycosyltransferase from a monofunctional precursor through the addition of an additional peptide has been described (48,49). The transition from the chitin homosaccharide polymer to the HA disaccharide polymer may utilize a comparable mechanism.

The beta-chain sugar polymers function as structural entities, and as such, must be resistant to degradation. Cellulose and chitin are the prime examples of such materials, which together with HA constitute the three major β-chain sugar polymers. In marked contrast, α-chain polymers are easily digested. They constitute sources of energy and energy reserves. Glycogen and starch are the ubiquitous materials in this category. They are composed of glucose units, but the simple change of a beta to an alpha bond constitute this fundamental switch. One need only spit, literally, on starch, and degradation begins, due to the amylase contained in saliva.

The putative precursor beta-chain sugar synthase gene gave rise to the three synthase genes that produce chitin, HA, and cellulose. As they become better understood, it will be possible to elucidate how the design of plant and animal cell wall composition, deposition and turnover came about (42). It is remarkable to consider that the cellulose of all of the three trillion trees of the forests worldwide must be synthesized by individual cells. A detailed description of the molecular biosynthesis of cellulose is available (50). Intriguingly, appendicularian, a tunicate, one of the earliest chordates, synthesizes cellulose (51).

Synthesis of sugar polymers may have occurred very early in evolution, and probably took place, as they do now, on the inner surface of the plasma membranes of those primitive cells. The synthesis of complex sugars was a requirement for the beginning of evolution of the organisms we know today (16), and are the likely precursors to the origin of life. The presence of ribose and deoxyribose sugar backbones in RNA and DNA polymers gives further credence to this hypothesis (52). A world of sugar polymers may well have preceded that of a DNA- and RNA-world, and was a necessary early step in the creation of life on this planet.

8. CHITINASES

Chitinases are the widely expressed hydrolases that cleave chitins, and are found in a wide variety of organisms. Chitinases have an important role in the decomposition of chitins, and potentially in the utilization of chitin as a major renewable resource with widespread

applications in medicine, agriculture, and industry. Yet, they are a relatively neglected family of enzymes. This remarkable considering that they are among the key enzymes of marine biology Their properties appear to be closely related to their biological functions, and have evolved widely in relation to those functions. In insects and crustaceans, chitinases digest the chitins that facilitate molting and other forms of ecdysis. Microorganisms produce chitinases in order to digest nutrients. Bacteria are one of the major sources of chitinases with enormous potential in industrial use.

An example of the potency of chitinases is found in the biology of krill. These tiny shrimp-like crustaceans congregate in large, dense, swarm-like masses in the oceans and are the major food source of baleen whales, sea birds, and other predators. When a spoonful of krill is warmed to room temperature, they expire, rapidly auto-digest, and the solution becomes water-clear, the chitin exoskeleton having become completely degraded by powerful chitinases. The turnover of the chitins in sea creatures amounts to 10 gigatons (10^{13} kg) per year. Without powerful chitinases, the oceans would turn murky. Yet, we know virtually nothing about these enzymes, so critical for marine biology, for the health of the seas, and ultimately, of the planet.

The properties of particular chitinases vary with their biological functions, and have evolved widely in relation to these functions. This is reflected in their wide range of molecular weights, from 30 kDa in plants and seaweeds to 120 kDa in mollusks and arthropods. Most vertebrate chitinases are members of the glycohydrolase 18 family of enzymes with an interesting parallel with the vertebrate hyaluronidase enzyme family. Vertebrate hyaluronidases are globular proteins that encompass a large groove or cleft that traverses the entire enzyme. They display a classical distorted $(alpha/beta)_8$ triose phosphate isomerase (TIM) barrel (53,36). The HA substrate fits perfectly within the groove of this barrel. It is tempting to assume that chitinases function in a parallel manner, that the chitin substrate fits within the groove of the chitinase barrel (54).

The family 19 chitinases are primarily from plant sources. Members of each family are homologues with similar amino acid sequences, but members of the two families are quite dissimilar. The plant chitinases have a major defensive role. In higher plants (55) and in seaweed (56), chitinases are a defense mechanism against pathogens, against fungi, and perhaps against insects.

Chitinase enzymes make up 50% of the soluble proteins in grapes. Their expression is relatively low initially and only begins to increase towards the end of the ripening period parallel to the rise of the level of sugars. This corresponds to the time when they are most vulnerable to fungal attacks (57). Chitinases are thus integral to the entire wine making industry.

9. CHITINASES IN VERTEBRATE BIOLOGY

Chitinases have now been identified in several vertebrate tissues including fish and mammals. The first human chitinase to be characterized was chitotriosidase, also known as chitinase 1, chit1, or ChT. This redundant nomenclature continues to confound the literature. Chitotriosidase has been isolated, cloned, and expressed (58,59). The human enzyme is on chromosome 1. It is a neutral-acting glycosyl hydrolase expressed in monocytes and macrophages (60,61).

Surprisingly, chitotriosidase is one of the most abundant proteins secreted by activated macrophages (62). This chitinase is expressed not only in monocytes/macrophages, but also in monocyte-derived cells such as Kupffer cells, osteoclasts, and dendritic cells (63). This enzyme may have a role in innate and acquired immunity as an increase in enzyme expression is observed in mature dendritic cells following an immune response (63).

A second mammalian chitinase is characterized by an acidic isoelectric point and is named acidic mammalian chitinase or AMCase (58). In rodents and man, this enzyme is abundant in the gastrointestinal tract and to a lesser extent in the lung. The extremely acidic pH optimum, consistent with its presence in the stomach, involves a histidine residue crucial to the active site, His187 (64).

Both the chitotriosidase chit1 or ChT, as well as AMCase are synthesized as a 50-kDa protein containing a 39-kDa N-terminal catalytic domain, a hinge region, and a C-terminal chitin-binding domain. In contrast to chit1, AMCase is extremely acid stable and has, in addition to a neutral pH activity, a distinct second pH optimum around pH 2. AMCase is capable of cleaving artificial chitin-like substrates as well as crab shell chitin and the chitin in fungal cell walls.

The open reading frame predicts a protein with a typical chitinase structure, including a signal peptide, a highly conserved catalytic domain and a chitin-binding domain. Recombinant expression of the cloned cDNA demonstrates that the encoded protein is secreted and has chitinolytic activity that is sensitive to the specific chitinase inhibitor, allosamidin, and that it also binds to chitin particles (65). Interestingly, such inhibition of chitinase activity promotes atherosclerosis in hyperlipidemic mice (66). There is obviously a great deal more to be learned about chitinases in human biology as will be discussed below.

The mammalian chitinase family 18 thus consists of these two members, chitinase-1 (ChT; chit1) and the acidic AMCase, and, as mentioned, is an ancient glycosyl hydrolase gene family that is widely expressed from prokaryotes to eukaryotes.

A long-standing formulation suggests that these enzymes arose because they provide evolutionary advantage as a protection against chitin-containing parasites and yeast infections (67). If this is correct, it is clear that they have many additional activities including immunological functions, most of which are unknown.

The evolutionary origin of these chitinases is of intrinsic interest considering that in a BLAST search, a 25% amino acid identity and 45% homology were found between the human and yeast chitinases (*Saccharomyces cerevisiae* S288c; NCBI Reference Sequence: NP_010659.1.) (A. Csóka, unpublished observations). Apparently, we have remnants of ancient yeast sequences contained in our genomes. This is possibly convergent evolution, or perhaps snippets of DNA were borrowed and incorporated into our own genome.

There have been very few studies on chitinases in lower animals. In one study, the first in an invertebrate, a single chitotriosidase-like gene from the amphioxus *Branchiostoma japonicum* was identified. This chitinase also consists of a signal peptide, a catalytic domain, a linker region and a chitin-binding domain. Sequence analysis shows that it is the common ancestor of both ChTs and AMCases, implicating that the two chitinases evolved from ancient gene duplication (68). This event may have occurred at about the time that HA emerged in evolution, as it separated from a probable chitin precursor. If so, it was a busy era.

Parallels have also been established between the processing of the predominant somatic hyaluronidase, HYAL1, and a chitinase. They are synthesized as 45 and 50 kDa proteins, respectively. This hyaluronidase and the chitinases exist as larger proteins, while lysosomes contain the 39 kDa proteolytically processed enzymes (69,70). Both size proteins are enzymatically active, so they do not represent inactive precursor zymogens and active enzyme products. The HYAL1 has an internal peptide of 25 amino acids that is removed requiring two separate proteolytic cleavage reactions. The two resulting fragments are presumably linked by disulfide bonds in order to maintain structure (69). It is tempting to invoke a similar scenario for the chitinase enzyme. The synthesis, sorting, and processing of human macrophage chitinase has been accomplished resulting in resolution of these various isoforms (70).

Many enzymes can be forced to catalyze the reverse of the normal reaction, by supplying an excess of the product, following the law of mass action. This is especially the case for hydrolytic enzymes that can synthesize polymers based on the monomers supplied. This may be a source of the chitins in vertebrate tissues, especially where chitinases occur. This is a major proviso and an additional explanation for the presence of chitins in vertebrates.

10. CHITINASE-LIKE PROTEINS, THE CHITIN-LECTINS

In humans, a number of chitinase-like proteins (CLPs) have been identified. Their functions are only beginning to be elucidated. Prominent among these is YKL-40, which has a major role in inflammation, tissue remodeling, and injury (17). Chitinases with enzymatic activity have a chitin-binding domain containing six cysteine residues responsible for chitin binding. In contrast, CLPs do not contain such chitin-binding domains, but still continue to bind to chitin with high affinity.

Following the gene duplication event described above, further duplication events occurred, followed by mutations leading to loss of chitinase activity, thereby allowing for the evolution of chitinase-lectins (64). A great number of studies have been performed describing their expression and differential regulation during specific immunologic reactions. Widespread gene specialization has occurred allowing for tissue-specific expression of chitinases and chitinase-lectins. To complicate this scenario, the profile of these proteins varies among different mammals suggesting relatively recent evolutionary events. This may explain why the mouse model for asthma is not relevant to the human disorder. Why are chitinases and CLPs such evolutionary hot spots?

It is evident that their function is crucial in bacterial infections and inflammatory diseases as well as in immune response regulation (71). This holds great promise for future research and applications.

11. CHITINASES AS BIOMARKERS FOR HUMAN DISEASE

Human chitinases and CLPs have been identified recently as biomarkers for several human diseases. ChT occurs in several lysosomal lipid storage diseases, the most prominent among which is Gaucher's disease, as reviewed below. The enzyme also occurs in sarcoidosis, and is used as a marker for disease severity (72). Sarcoidosis is a multisystemic disorder of unknown origin marked by wide spread granulomas. ChT also plays a pivotal role in the context of several infectious diseases including malaria (73) and fungal infections. It is also involved in such disparate disorders as thalassemia (74) and visceral Leishmaniasis (75).

Monitoring enzyme activity in these disorders as a diagnostic tool during progression, and as a marker for therapeutic response requires sensitive enzyme assays. However, quantification is complicated by chitin substrate inhibition. Such inhibition of ChT by substrate can now be attributed in part to an attendant transglycosylation reaction (76). It would be of intrinsic interest to establish

whether this parallels the transglycosyation reaction characteristic of the hyaluronidases (77,78).

The AmCase enzyme is associated with the pathogenesis of asthma, and has been explored in a mouse model. Inhibition of AmCase alleviates the disorder and its inflammatory-driven pathology (58,79). However, it is critical to point out, as aforementioned, major differences in chemokine response profiles and recent evolutionary tissue-specific chitinase mutations between mice and humans, and in particular, between their myeloid cells (80).

Studies of spatial and temporal expression patterns of chitinase genes in the developing zebra fish reveal six chitinases and chitinase-like proteins, all members of the glycoside hydrolase family 18. Elucidating the functions of each of these proteins promises to be a potent tool for future analyses of their various functions and may provide insight into their participation in human disease (81,82)

12. GAUCHER'S DISEASE

Gaucher's disease is an inherited disorder of glucocerebroside metabolism, a prototypical lysosomal storage disease. The disorder results in widespread accumulation of macrophages engorged with predominantly lysosomal glucocerebroside. This results in enlargement of liver and spleen, the usual presenting physical findings. Other tissues with a large presence of cells from the monocyte lineage are also predominantly affected such as in bone marrow and the lungs. Other presenting complaints can include joint pain and skeletal weakness with avascular osteonecrosis. Patients also have easy bruising, fatigue, anemia, and mild thrombocytopenia. However, severity of symptoms varies widely between patients.

The disorder is attributed to a mutation on chromosome 1 (1q22), and affects men and women equally. The disease is caused by defect in a housekeeping gene product, lysosomal glucocerebrosidase, also called beta-glucosidase, and is the most common of the lysosomal storage diseases. This enzyme catalyzes the breakdown of glucosylceramide. The waste products from defective breakdown are taken up by macrophages, which are unable to degrade them further. These cells become bloated with undigested material, turning them into foamy Gaucher cells. The intracellular material has the appearance of crumpled tissue paper.

An inexplicable observation is that patients with Gaucher's disease have a 1,000- fold increase in circulating levels of ChT. The activity is used as a disease marker and in clinical management of the disorder (64,83,84). Why there is this association between beta-glucosidase expression (or glucocerebrosidase) and ChT is a mystery. Some of this enigma might be explored in cultured monocytes isolated from the blood stream of Gaucher's disease patients. Do they synthesize chitinase in addition to the defective glycocerebrosidase? Is there cross talk between expression of the two proteins? Do these cells synthesize chitins? How do these parameters compare to monocytes obtained from Gaucher's disease gene carriers and from normal control patients?

13. CHITIN OLIGOMERS AT THE INITIATION SITE OF HYALURONAN SYNTHESIS

Hyaluronan, as a probable evolutionary descendant of chitin, utilizes the chitin platform apparently for its own synthesis. The non-reducing ends of HA polymers contain seven to nine N-acetyl-glucosamine residues. These function as primers for the subsequent activity of the HA synthase enzymes (85,86).

However, it should be pointed out that under these *in vitro* conditions, chitin oligosaccharides are found at the non-reducing end of the growing HA chain only when the enzyme is deprived of UDP-glucuronic acid, and is not found under normal cellular conditions.

Another feature of this model is that the initiating chitin oligosaccharide, with its inability to attract water, can act as a stiff needle that pierces itself through the plasma membrane, creating a pathway for the voluminously hydrated HA molecule to follow. The initial chitin needle can facilitate the subsequent passage of the attached flexible HA thread through the plasma membrane and its exit from inside the cell into the extracellular matrix. There may be considerable accuracy in this needle and thread analogy.

Hyaluronan turns over very rapidly in vertebrate tissues as a result of the hyaluronidase family of enzymes. These are extremely efficient enzymes with catalytic rates one log greater than conventional vertebrate enzymes (36). The HA components of the initiating HA chains are easily degraded by hyaluronidases, but the small chitin oligomers at the origins of the lengthy HA chains presumably remain relatively intact. Vertebrate chitinases exist, but are sparse and far less active. With time, chitin oligomers can accumulate, among the processes that may occur with aging. Most tissues can withstand such buildup of chitin fragments, but they may be particularly toxic to neurons. Such toxicity has been documented *in vitro*, in neuron cell cultures (87). The presence of chitin fragments in Alzheimer disease brains has now been documented (88-90). Such chitinous deposits are present in the brains of a sporadic form of Alzheimer's disease, but not in the more rare familial form. The putative toxicity of these chitin fragments in the brain may contribute to the dementia that characterizes Alzheimer's disease patients.

14. THE HYALURONAN VERSUS CHITIN PRODUCTS OF DG42 DICHOTOMY RESOLVED

The wave of DG42 activity that occurs during frog embryogenesis between mid-blastula and neurulation may be a reflection of copious synthesis of HA. It is more difficult to initiate new chains than to extend existing chains of HA. The DG42 activity may reflect enormous amounts of new HA chain initiation. As discussed, these chitin chains begin as a run of seven to nine monosaccharide sugars, before the disaccharide HA chain synthesis is initiated. The experiments that documented either chitin or HA synthesis are both correct. Depending on how the experiments were constructed, it was possible to identify either a burst of chitin synthesis (1) or of HA synthesis (4) with equal validity (5).

A similar phenomenon might be observed in developing zebra fish embryos (2,81). Details of such a chemical chimeric switch could now be analyzed in greater detail in this more tractable system.

15. CHITIN, CHITINASES, AND CHITINASE-LIKE PROTEINS IN IMMUNE RECOGNITION AND IN MALIGNANCY

Chitinases and CLPs are well conserved among all vertebrates and appear to be involved in immune recognition (82). Though chitin oligomers occur in vertebrate tissues, it is the longer chitinous chains that may be the objects of immune recognition; another example of size-dependent functions of these simplest of homopolymers (91). The degradation of the longer chains is mediated by immune-recognition, followed by a complex series of steps in immune-stimulation that precedes the degradation by chitinases. Both chitinases and CLP's are involved in the process. As is often the case in immune-recognition, the reaction can be overly zealous, and may in itself produce pathology.

An excellent overview of the interplay between chitin, immune-recognition, chitinases, CLP's and human pathology involving asthma, bacterial infections, exposure to chitinous parasites, and even cancer signaling was provided recently (82). The biomedical applications of chitin and chitinases in a variety of fields, including food technology, material science, microbiology, agriculture, wastewater treatment, drug delivery systems, tissue engineering, and nanotechnology have also been reported (10,11).

A consistent observation is that some chitins and chitin-containing glycans are expressed in cancer cells that do not occur in normal cells. They are associated with metastasis and poor prognosis in many cancers (92). Sulfated derivatives of chitin have the ability to inhibit tumor progression and metastatic spread (93,94). Sulfated HA derivatives have similar properties (95). These observations could be the basis for formulating new chemotherapeutic drugs.

16. CHITIN IN ALZHEIMER'S DISEASE

Alzheimer's disease is a fatal neurodegenerative disease that begins with subtle memory impairment, and progresses to dementia and complete debilitation. It is the most common of the senile dementias. The distinct brain lesions include neurofibrillary tangles within neuronal cells and senile plaques outside of cells, referred to by Pathologists as "spaghetti and meatballs".

The amyloid plaques of Alzheimer's disease have been shown to contain chitin (87-89). Such deposits are present in sporadic cases of Alzheimer's disease but not in familial cases.

In cell culture studies, incubation of neuronal cell lines with the sugar that comprises chitin, GlcNAc and its precursor UDP-GlcNAc induces significant cell death. Similar results are obtained with primary cultures of hippocampal neurons and in hippocampal slices. The accumulation of chitin-like oligomers in such cultures and tissue slices has been confirmed. Phagocytosis of the chitin chains by microglia is also observed (89).

A testable hypothesis would suggest that the small run of N-acetyl glucosamine residues, or minute chitin-like fragments that precede starts of new HA chains accumulate in the vertebrate body. The HA turns over rapidly, secondary to the action of the potent somatic hyaluronidases (36). However, the initiating chitin fragments may accumulate. They are tolerated in much of the body, but may be toxic to neurons. Accumulation continues over years and eventually may trigger the cognitive decline that characterizes Alzheimer's disease patients. The onset however must certainly occur years before there are actual changes in mental status.

The important over-arching theme here is that the initiating chitin oligomers accumulate because they are difficult to degrade. They persist and may be particularly toxic to neurons, the possible basis of several degenerative neuronal disorders, particularly Alzheimer's disease.

Addition evidence that supports the role of chitins in Alzheimer's disease, are the observations that both chitotriosidase and the chitin-binding protein, YKL-40, are elevated on the cerebrospinal fluid of Alzheimer's disease patients (96,97). Also, plasma level of chitinase 3-like 1 (CH3L1) protein increases in patients with Alzheimer's disease (98).

A recent caveat in our understanding of the role of chitin in Alzheimer's disease is the documentation of fungal infections. Proteomic analysis provides evidence for the existence of fungal proteins in brain samples from

Alzheimer's disease patients, but not in control samples. PCR analysis reveals a variety of fungal species are involved (99). Fungal proteins and DNA were found in the brains and neurovascular tissue of all eleven AD patients examined and none in control patients (100). Thus an alternative explanation for the presence of chitin in AD is that there is an exogenous source in the form of fungal infections. These studies are further confirmed by direct visualization using a variety of anti-fungal antibodies (101). This exogenous source provides an alternate explanation for chitins in the brains of patients with Alzheimer's disease.

17. INITIATION OF ALL SUGAR POLYMERS; POSSIBLE PARALLELS WITH GLYCOGEN'S GLYCOGENIN

Glycogenin is a protein involved in the initiation of new glycogen chains. It is relatively easy to elongate existing chains of glycogen, but very difficult to initiate the synthesis *de novo* of new glycogen molecules. To accomplish the task of initiating new chains, a protein termed glycogenin is utilized, particularly abundant in tissues with dense concentrations of glycogen deposition such as muscle and liver (102). One glycogenin molecule is covalently bound to each mature molecule of glycogen. Glycogenin initiates the first step in the synthesis of glycogen by self- glycosylation of short eight to 12 glucose residues. Glucose is covalently bound to a tyrosine in the glycogenin protein, the initiation site of this self-glycosylation reaction (103). After this initiating reaction, glycogen synthase extends this sugar primer to form, together with the branchng enzymes, the glycogen molecule (104,105).

The short run of glucose residues bears a remarkable and striking parallel with the chitin sugars that precede HA synthesis. This may represent a general biological phenomenon overlooked heretofore, that polymer sugars have a common theme in the initiation of their synthesis, the use of a unique class of jump-start proteins, the "genins".

A deficiency of glycogenin has been reported in patients resulting in a depletion of glycogen (106, 107), the polar opposite of a glycogen storage disease. It is a glycogen deficiency disease, a new class of human genetic disorders.

The hypothesis promulgated here is that all sugar homopolymers, whether of the alpha- or beta-variety, are easy to elongate, but difficult to initiate. They may all parallel the glycogenin strategy in their initiation. Perhaps there is a protein involved in the initiation of chitin synthesis that is yet to be discovered. This putative protein could be named "chitinogenin". A parallel molecule might exist for the initiation of HA that could be dubbed "hyaluronogenin". A further testable assumption might be that there is a high degree of homology between chitinogenin and hyaluronogenin, products of ancient gene duplication. Presumably, in parallel with the glycogenin strategy, there is the attachment of a single N-acetylglucosamine to a hydroxyl group of an amino acid within such initiator proteins. Several such sugars are then added, a probable run of seven to nine sugars, before the synthases take over to extend these chains.

If such a scenario does hold, it would be possible to determine whether even chitin synthesis in yeast follows this pattern, and whether there are sequence homologies between the putative chitinous glycogenin-like molecule in yeast, arthropods, insects and vertebrates. This is given credence by the observation that mammalian, bacterial and yeast glycogen synthases all use this self-glycosylating glycogenin primer (108).

It would also be of intrinsic interest to establish whether cellulose follows such a pattern, and that a putative protein initiates cellulose polymer synthesis, "cellulogenin". The one for pectin would be termed "pectinogenin" if indeed this were a universal mechanism for the initiation of long sugar chain synthesis in plants and animals. The vast variety of plant gum and resin polymers could follow a similar mode of initiation. This has enormous bio-industrial and commercial potential.

18. A JEWISH CONNECTION

Gaucher's disease occurs in the Ashkenazi Jewish population at a rate 100 times that in the general population, one in 400 births as opposed to one in 40,000 births. The recessive gene exists in that Jewish population at a much greater incidence. The Hardy-Weinberg law of genetics estimates that nearly 10% of Ashkenazi Jews are carriers of the recessive gene for Gaucher's disease. The levels of chitinase activity in carriers of Gaucher's disease should be made, to document that this carrier population has a higher level of circulating chitinase. Such activity would be available for the digestion of toxic chitin oligomers and for a generally decreased level of chitin-induced neuronal toxicity and the attendant progressive dementia.

It would be important to establish whether the heterozygote carriers of the Gaucher's disease gene have a lower incidence of Alzheimer's disease compared to age-matched controls. If such a difference could be documented, it would justify using human recombinant chitinase as a treatment modality for Alzheimer's disease, particularly in its early stages. Alternatively, autologous tissue transplantation following chitinase gene transfection could be considered.

19. CONCLUSION

As shown here, chitins, chitin-binding proteins, chitinases, chitinase-lectins, and perhaps even chitin synthases have fundamental roles in a variety of

vertebrate systems. Their ubiquitous presence suggests they have functions that are more complex and far more diverse than previously realized. A chitin synthase gene should be identified in the genome of some vertebrates. Do human chitinases have hyaluronidase activity? Can experiments with cultures of isolated human monocytes from Gaucher's disease patients elucidate the interactions between cerebroside and chitinase expression? Do Gaucher's cells synthesize increased levels of chitins, or HA, or both? Further documentation that accumulation of chitin fragments may contribute to the dementia of Alzheimer's disease is urgently needed. And finally, the possibility of a chitinase treatment for the disease is of the utmost importance. An over-arching biological scheme for chitins and chitin-related enzymes and proteins in vertebrate biology remains to be formulated. Obviously, much interesting research lies ahead.

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21. REFERENCES

1. Semino, C.E., and P.W. Robbins: Synthesis of "Nod"-like chitin oligosaccharides by the Xenopus developmental protein DG42. *Proc Natl Acad Sci U S A* 92, 3498-3501 (1995) DOI: 10.1073/pnas.92.8.3498
2. Semino, C.E., Specht, C.A., Raimondi, A., and P.W. Robbins: Homologs of the Xenopus developmental gene DG42 are present in zebrafish and mouse and are involved in the synthesis of Nod-like chitin oligosaccharides during early embryogenesis. *Proc Natl Acad Sci U S A.* 93, 4548-4553 (1996) DOI: 10.1073/pnas.93.10.4548
3. Rosa, F., Sargent, T.D., Rebbert, M.L., Michaels, G.S., Jamrich, M., Grunz, H., Jonas, E, Winkles, J.A., and I.B. Dawid: Accumulation and decay of DG42 gene products follow a gradient pattern during Xenopus embryogenesis. *Dev Biol* 129, 114-123 (1988) DOI: 10.1016/0012-1606(88)90166-2
4. Meyer, M.F., and G. Kreil: Cells expressing the DG42 gene from early Xenopus embryos synthesize hyaluronan. *Proc Natl Acad Sci U S A* 93, 4543-4547 (1986) DOI: 10.1073/pnas.93.10.4543
5. Varki, A.: Does DG42 synthesize hyaluronan or chitin? A controversy about oligosaccharides in vertebrate development. *Proc Natl Acad Sci U S A* 93, 4523-4525 (1996) DOI: 10.1073/pnas.93.10.4523
6. Bo, M., Bavestrello, G., Kurek, D., Paasch, S., Brunner, E., Born, R., Galli, R., Stelling A.L., Sivkov, V.N., Petrova, O.V., Vyalikh, D., Kummer, K., Molodtsov, S.L., Nowak, D., Nowak, J., and H.Ehrlich: *Int J Biol Macromol* 51, 129-137 (2012) DOI: 10.1016/j.ijbiomac.2012.04.016
7. Dunn, D.F., and M.H. Liberman: Chitin in sea anemone shells. *Science* 221, 157-159 (1983) DOI: 10.1126/science.221.4606.157
8. Muzzarelli, R. A.A. Native, industrial and fossil chitins, In: Chitin and Chitinases. Eds: P. Jollès, RAA Muzzarelli, Berkhäuser Verlag, Basel (1999) DOI: 10.1007/978-3-0348-8757-1_1
9. Lenardon, M.D., Carol, A., Munro, C.A, and N.A.R.Gow: Chitin synthesis and fungal pathogenesis. *Curr Opin Microbiol* 13, 416–42 (2010) DOI: 10.1016/j.mib.2010.05.002
10. Khoushab, F., and M. Yamabhai: Chitin research revisited. *Mar Drugs* 8, 1988-2012 (2010) DOI: 10.3390/md8071988
11. Khoushab, F., Jaruseranee, N., Tanthanuch, W., and M. Yamabhai: Formation of chitin- based nanomaterials using a chitin-binding peptide selected by phage-display. *Int J Biol Macromol* 50, 1267-1274 (2012) DOI: 10.1016/j.ijbiomac.2012.03.016
12. Periayah, M.H., Halim, A.S., and A.Z. Saad: Chitosan: A Promising Marine Polysaccharide for Biomedical Research. *Pharmacogn Rev* 10, 39-42 (2016) DOI: 10.4103/0973-7847.176545
13. Yang, R., Li, H., Huang, M., Yang, H., and A. Li: A review on chitosan-based flocculants and their applications in water treatment. *Water Res* 95, 59-89 (2016) DOI: 10.1016/j.watres.2016.02.068
14. Merzendorfer, H.:. The cellular basis of chitin synthesis in fungi and insects: common principles and differences. *Eur J Cell Biol* 90, 759-769 (2011) DOI: 10.1016/j.ejcb.2011.04.014
15. Klute, M.J., Melançon, P., and J.B. Dacks: Evolution and diversity of the Golgi. *Cold Spring Harb Perspect Biol* 3,1–14 (2011)

DOI: 10.1101/cshperspect.a007849

16. Varki, A.: Evolutionary forces shaping the Golgi glycosylation machinery; Why cell surface glycans are universal to living cells. *Cold Spring Harb Perspect Biol* 3, 1–14 (2011) DOI: 10.1101/cshperspect.a005462
17. Lee, J.Y., Choi, B., Wu, B., and M. Lee: Customized biomimetic scaffolds created by indirect three-dimensional printing for tissue engineering. *Biofabrication* 5, 045003 (2013) DOI: 10.1088/1758-5082/5/4/045003
18. Gupta, B., Arorab, A., Saxena, S., and M.S. Alam: Preparation of chitosan– polyethylene glycol coated cotton membranes for wound dressings: preparation and characterization. *Polymers for Advanced Technologies* 20, 58–65 (2008) DOI: 10.1002/pat.1280
19. You, Y., Park, W.H., Ko, B.M., and B.M. Min: Effects of PVA sponge containing chitooligosaccharide in the early stage of wound healing. *J Mater Sci Mater Med* 15, 297–301 (2004) DOI: 10.1023/B:JMSM.0000015491.91918.64
20. Azuma, K., Izumi, R., Osaki, T., Ifuku, S., Morimoto, M., Saimoto, H., Minami, S., and Y. Okamoto: Chitin, chitosan, and its derivatives for wound healing: old and new materials. *J Funct Biomater* 6, 104-142 (2015) DOI: 10.3390/jfb6010104
21. Izumi, R., Komada, S., Ochi, K., Karasawa, L., Osaki, T., Murahata, Y., Tsuka, T., Imagawa T., Itoh, N., Okamoto, Y., Izawa, H., Morimoto, M., Saimoto, H, Azuma, K., and S. Ifuku: Favorable effects of superficially deacetylated chitin nanofibrils on the wound healing process. *Carbohydr Polym* 123, 461-467 (2015) DOI: 10.1016/j.carbpol.2015.02.005
22. Choi, B.K., Kim, K.Y. Yoo, Y.J., Oh, S.J.;Choi, J.H.and C. Y. Kim: *In vitro* antimicrobial activity of a chitooligosaccharide mixture against Actinobacillus actinomycetemcomitans and Streptococcus mutans. *Int J Antimicro Agents* 18, 553–557 (2001) DOI: 10.1016/S0924-8579(01)00434-4
23. Tang, W.J., Fernandez, J.G., Sohn, J.J., and C.T. Amemiya: Chitin is endogenously produced in vertebrates. *Curr Biol* 25, 897-900 (2015) DOI: 10.1016/j.cub.2015.01.058
24. Bakkers, J, Kijne, J.W., and H.P. Spaink: Function of chitin oligosaccharides in plant and animal development. *EXS* 87, 71-83 (1999) DOI: 10.1007/978-3-0348-8757-1_5
25. Spicer, A.P., and J.Y. Tien: Hyaluronan and morphogenesis. Birth Defects Res C Embryo Today 72, 89-108 (2004) DOI: 10.1002/bdrc.20006
26. Haylock, D.N. and S.K. Nilsson: The role of hyaluronic acid in hemopoietic stem cell biology. *Regen Med* 1, 437-445 (2006) DOI: 10.2217/17460751.1.4.437
27. Al-Hajj, M., and M.F.Clarke: Self-renewal and solid tumor stem cells. *Oncogene* 23, 7274-7282 (2004) DOI: 10.1038/sj.onc.1207947
28. Csóka, A.B., and R. Stern: Hypotheses on the evolution of hyaluronan: a highly ironic acid. *Glycobiology* 23, 398-411 (2013) DOI: 10.1093/glycob/cws218
29. DeAngelis, P.L.: Molecular directionality of polysaccharide polymerization by the *Pasteurella multocida* hyaluronan synthase. J Biol Chem 274, 26557-26562 (1999) DOI: 10.1074/jbc.274.37.26557
30. Ehrlich, H., Rigby, J.K., Botting, J.P., Tsurkan, M.V., Werner, C., Schwille, P., Petrášek, Z., Pisera, A., Simon, P., Sivkov, V.N., Vyalikh, D.V., Molodtsov, S.L., Kurek, D., Kammer, M., Hunoldt, S., Born, R., Stawski, D., Steinhof, A., Bazhenov, V.V., and T. Geisler: Discovery of 505-million-year old chitin in the basal demosponge Vauxia gracilenta. *Sci Rep* 3, 349 (2013) DOI: 10.1038/srep03497
31. Itano, N., and K. Kimata: Expression cloning and molecular characterization of HAS protein, a eukaryotic hyaluronan synthase. *J Biol Chem* 271, 9875–9878 (1996) DOI: 10.1074/jbc.271.17.9875
32. Weigel, P.H., Hascall, V.C., and M. Tammi: Hyaluronan synthases. *J Biol Chem* 1272, 13997–14000 (1997) DOI: 10.1074/jbc.272.22.13997
33. Weigel P.H.: Hyaluronan Synthase: The Mechanism of Initiation at the Reducing End and a Pendulum Model for Polysaccharide Translocation to the Cell Exterior. *Int J Cell Bio* 2015, 367579 (2015) DOI: 10.1155/2015/367579

34. Hascall, V.C., Majors, A.K., De La Motte, C.A., Evanko, S.P., Wang, A., Drazba, J.A, Strong, S.A., and T.N. Wight: Intracellular hyaluronan: a new frontier for inflammation? *Biochim Biophys Act* 1673, 3-12 (2004)
DOI: 10.1016/j.bbagen.2004.02.013

35. Camenisch, T.D., Spicer, A.P., Brehm-Gibson, T., Biesterfeldt, J., Augustine, M.L, Calabro, A. Jr, Kubalak, S., Klewer, S.E., and J.A.McDonald:. Disruption of hyaluronan synthase-2 abrogates normal cardiac morphogenesis and hyaluronan-mediated transformation of epithelium to mesenchyme. *J Clin Invest* 106, 349-360 (2000)
DOI: 10.1172/JCI10272

36. Stern, R., and M. Jedrzejas: Hyaluronidases: their genomics, structures, and mechanisms of action. *Chem Review* 106, 818 -839 (2006)
DOI: 10.1021/cr050247k

37. Venkatachalam, K.V.: Human 3′-phosphoadenosine 5′-phosphosulfate (PAPS) synthase: Biochemistry, molecular biology and genetic deficiency. *IUBMB Life* 55, 1–11 (2003)
DOI: 10.1080/1521654031000072148

38. Siczkowski, M., Andrew, T., Amos, S., and M.Y. Gordon: Hyaluronic acid regulates the function and distribution of sulfated glycosaminoglycans in bone marrow stromal cultures. *Exp Hematol* 21, 126-130 (1993)

39. Bevan, M., and S. Walsh: The Arabidopsis genome: a foundation for plant research. *Genome Res* 15, 1632-1642 (2005)
DOI: 10.1101/gr.3723405

40. Somerville, C.C. and R.R. Colwell: Sequence analysis of the beta-N-acetylhexosaminidase gene of Vibrio vulnificus: evidence for a common evolutionary origin of hexosaminidases. *Proc Natl Acad Sci U S A* 90, 6751-6755 (1993)
DOI: 10.1073/pnas.90.14.6751

41. Spicer, A.P., and J.A. McDonald: Characterization and molecular evolution of a vertebrate hyaluronan synthase gene family. *J Biol Chem* 273, 1923–1930 (1998)
DOI: 10.1074/jbc.273.4.1923

42. Richmond, T.A., and C.R.Somerville: The cellulose synthase superfamily. *Plant Physiol* 124, 495–498 (2000)
DOI: 10.1104/pp.124.2.495

43. Merz, R.A., Horsch, M., Nyhlén, L.E., and D.M. Rast: Biochemistry of chitin synthase. In: Chitin and Chitinases. Eds: P. Jollès and R.A.A. Muzzarelli. Birkhäauser Verlag, Basel (1999)
DOI: 10.1007/978-3-0348-8757-1_2

44. Merzendorfer, H., and L. Zimoch: Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinases. *J Exp Biol* 206, 4393-4412 (2003)
DOI: 10.1242/jeb.00709

45. Zakrzewski, A.C., Weigert, A., Helm, C., Adamski, M., Adamska, M., Bleidorn, C., Raible, F., and H. Hausen: Early divergence, broad distribution, and high diversity of animal chitin synthases. *Genome Biol Evol* 6, 316-325 (2014)
DOI: 10.1093/gbe/evu011

46. Basha, A., Basha, F., Ali, S.K., Hanson.,PR., Mitscher, L.A., and B.R. Oakley: Recent progress in the chemotherapy of human fungal diseases. Emphasis on 1,3-β-glucansynthase and chitin synthase inhibitors. *Curr Med Chem* 2, 4859-4887 (2013)
DOI: 10.2174/092986732038131128102220

47. Chaudhary, P.M., Tupe, S.G., and M.V. Deshpande: Chitin synthase inhibitors as antifungal agents. Mini Rev Med Chem 13, 222-236 (2013)
DOI: 10.2174/1389557511313020005

48. Sobhany, M., Kakuta, Y., Sugiura, N., Kimata, K., and M. Negishi: The chondroitin polymerase K4CP and the molecular mechanism of selective bindings of donor substrates to two active sites. *J Biol Chem* 283, 32328–32333 (2008)
DOI: 10.1074/jbc.M804332200

49. Sobhany, M., Kakuta, Y., Sugiura, N., Kimata, K., and M. Negishi: The structural basis for a coordinated reaction catalyzed by a bifunctional glycosyltransferase in chondroitin biosynthesis. *J Biol Chem* 287, 36022–36028 (2012)
DOI: 10.1074/jbc.M112.375873

50. McNamara J.T., Morgan, J.L., and J. Zimmer: A molecular description of cellulose biosynthesis. *Annu Rev Biochem* 84, 895-921 (2015)
DOI: 10.1146/annurev-biochem-060614-033930

51. Kimura, S., Ohshima, C., Hirose, E., Nishikawa, J., and T. Itoh: Cellulose in the house of the appendicularian Oikopleura

rufescens. *Protoplasma* 216, 71-74 (2001)
DOI: 10.1007/BF02680133

52. Stern, R., and J.M. Jedrzejas: Carbohydrate polymers at the center of life's origins: the importance of molecular processivity. *Chem Rev* 108, 5061-5085 (2008)
DOI: 10.1021/cr078240l

53. Rigden, D.J., Jedrzejas, M.J., and L.V.de Mello: Identification and analysis of catalytic TIM barrel domains in seven further glycoside hydrolase families. *FEBS Lett* 544, 103-111 (2013)
DOI: 10.1016/S0014-5793(03)00481-2

54. Jedrzejas, M.J., and R. Stern: Structures of vertebrate hyaluronidases and their unique mechanisms of hydrolysis. *Proteins* 61, 227-238 (2005)
DOI: 10.1002/prot.20592

55. Stintzi, A., Heitz, T., Prasad, V., Wiedemann-Merdinoglu, S., Kauffmann, S., Geoffroy, P., Legrand, M, and B. Fritig: Plant 'pathogenesis-related' proteins and their role in defense against pathogens. *Biochimie* 75, 687-706 (1993)
DOI: 10.1016/0300-9084(93)90100-7

56. Sekiguchi, J. Matsumiya, M. and A. Mochizuki: Ditribution of chitinolytic enzymes in seaweeds. *Fisheries Sci* 61, 876-881 (1995)

57. Waters, E.J., Hayasaka, Y., Tattersall, D.B., Adams, K.S., and P.J. Williams: Sequence analysis of grape (Vitus vinafera) berry chitinases that cause haze formation in wines. *J Agric Food Chem* 46, 4950-4957 (1998)
DOI: 10.1021/jf980421o

58. Boot, R.G., Bussink, A.P., Verhoek, M., de Boer, P.A., Moorman, A.F., and J.M. Aerts: Marked differences in tissue-specific expression of chitinases in mouse and man. *J Histochem Cytochem* 53, 1283-1292 (2005)
DOI: 10.1369/jhc.4A6547.2005

59. Boot, R.G., Blommaart, E.F., Swart, E., Ghauharali-van der Vlugt, K., Bijl, N., Moe, C., Place, A., and J.M. Aerts: Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. *J Biol Chem* 276, 6770-6778 (2001)
DOI: 10.1074/jbc.M009886200

60. Boot, R.G., Renkema, G.H., Strijland, A., van Zonneveld, A.J., and J.M. Aerts: Cloning of a cDNA encoding chitotriosidase, a human chitinase produced by macrophages. *J Biol Chem* 270, 26252–26256 (19995)

61. Escott, G.M., and D.J. Adams: Chitinase activity in human serum and leukocytes. *Infect Immun* 163, 4770–4773 (1995)

62. Malaguarnera L.: Chitotriosidase: the yin and yang. *Cell Mol Life Sci* 63, 3018-3029 (2006)
DOI: 10.1007/s00018-006-6269-2

63. Di Rosa, M., Tibullo, D., Cambria, D., Distefano, G., Saccone, S., Di Raimondo F, and L. Malaguarnera: Chitotriosidase Expression during Monocyte-Derived Dendritic Cells Differentiation and Maturation. *Inflammation* 38:2082-2091 (2015)
DOI: 10.1007/s10753-015-0190-5

64. Bussink, A.P., Vreede., J, Aerts, J.M, and R.G. Boot: A single histidine residue modulates enzymatic activity in acidic mammalian chitinase. *FEBS Lett* 582, 931-935 (2008)
DOI: 10.1016/j.febslet.2008.02.032

65. Huang, G.: Recent progress on synthesis and activities of allosamidin and its analogues. *Mini Rev Med Chem* 12, 665-670 (2012)
DOI: 10.2174/138955712800626700

66. Kitamoto, S., Egashira, K., Ichiki, T., Han, X., McCurdy, S., Sakuda, S., Sunagawa, K., and W.A. Boisvert: Chitinase inhibition promotes atherosclerosis in hyperlipidemic mice. *Am J Pathol* 183, 313-325 (2013)
DOI: 10.1016/j.ajpath.2013.04.003

67. Wiesner, D.L., Specht, C.A., Lee, C.K., Smith, K.D., Mukaremera L, Lee, S.T., Lee, C.G., Elias,J.A, Nielsen, J.N., Boulware, D.R., Bohjanen, P.R., Jenkins, M.K., Levitz, S.M., and K. Nielsen: Chitin recognition via chitotriosidase promotes pathologic type-2 helper T cell responses to cryptococcalinfection. *PLoS Pathog* 12, 11 (2015)

68. Xu, N., and S. Zhang: Identification, expression and bioactivity of a chitotriosidase-like homolog in amphioxus: dependence of enzymatic and antifungal activities on the chitin-binding domain. *Mol Immunol* 51, 57-65 (2012).
DOI: 10.1016/j.molimm.2012.02.003

69. Csóka, A.B., Frost, G.I., Wong, T., and R. Stern: Purification and microsequencing of hyaluronidase isozymes from human urine. *FEBS Lett* 417, 307-310 (1997)
DOI: 10.1016/S0014-5793(97)01309-4

70. Renkema, G.H., Boot, R.G., Strijland, A., Donker-Koopman, W.E., van den Berg, M., Muijsers, A.O., and J.M. Aerts: Synthesis, sorting, and processing into distinct isoforms of human macrophage chitotriosidase. *Eur J Biochem* 244, 279-285 (1997)
DOI: 10.1111/j.1432-1033.1997.00279.x

71. Di Rosa, M., Distefano, G., Zorena, K., and L. Malaguarner: Chitinases and immunity: Ancestral molecules with new functions. *Immunology* 221, 399-411 (2016)
DOI: 10.1016/j.imbio.2015.11.014

72. Bargagli, E., Bennett, D., Maggiorelli, C., Di Sipio, P., Margollicci, M., Bianchi, N, and P. Rottoli: Human chitotriosidase: a sensitive biomarker of sarcoidosis. *J Clin Immunol* 33, 264-270 (2013)
DOI: 10.1007/s10875-012-9754-4

73. Erdman, L.K., Petes, C., Lu, Z., Dhabangi, A., Musoke, C., Cserti-Gazdewich, C.M., Lee, C.G, Liles, W.C., Elias, J.A., and K.C. Kain: Chitinase 3-like 1 is induced by Plasmodium falciparum malaria and predicts outcome of cerebral malaria and severe malarial anaemia in a case-control study of African children. *Malar J* 13, 279 (2014)
DOI: 10.1186/1475-2875-13-279

74. Barone, R., Sotgiu, S., and S. Musumeci: Plasma chitotriosidase in health and pathology. *Clin Lab* 53, 321-333 (2007)

75. Dostálová, A., and P. Volf: Leishmania development in sand flies: parasite-vector interactions overview. *Parasit Vectors* 5, 276-278 (2012)
DOI: 10.1186/1756-3305-5-276

76. Aguilera, B., Ghauharali-van der Vlugt, K., Helmond, M.T., Out, JM, Donker-Koopman, W.E., Groener, J.E., Boot, R.G., Renkema, G.H., van der Marel, G.A., van Boom, J.H, Overkleeft, H.S., and J.M. Aerts: Transglycosidase activity of chitotriosidase: improved enzymatic assay for the human macrophage chitinase. 278, 40911-40916 (2003)

77. Hoffman. P., Meyer, K., and A. Linker: Transglycosylation during the mixed digestion of hyaluronic acid and chondroitin sulfate by testicular hyaluronidase. *J Biol Chem* 219, 653-663 (1956)

78. Saitoh, H., Takagaki, K., Majima, M., Nakamura, T., Matsuki, A., Kasai, M., Narita, H., and M. Endo: Enzymic reconstruction of glycosaminoglycan oligosaccharide chains using the transglycosylation reaction of bovine testicular hyaluronidase. *J Biol Chem* 270, 3741-3747 (1995)
DOI: 10.1074/jbc.270.8.3741

79. Matsumoto, T., Inoue, H., Sato, Y., Kita, Y., Nakano, T., Noda, N., Eguchi-Tsuda, M., Moriwaki, A., Kan-O, K., Matsumoto, K., Shimizu, T., Nagasawa, H., Sakuda, S., and Y. Nakanishi: Demethylallosamidin, a chitinase inhibitor, suppresses airway inflammation and hyperresponsiveness. *Biochem Biophys Res Commun* 390, 103-108 (2009)
DOI: 10.1016/j.bbrc.2009.09.075

80. Raes, G., Van den Bergh, R., De Baetselier, P., Ghassabeh, G.H., Scotton, C., Locati, M., Mantovani, A., and S. Sozzani: Arginase-1 and Ym1 are markers for murine, but no human, alternatively activated myeloid cells. *J Immunol* 174, 6561-6562 (2005)
DOI: 10.4049/jimmunol.174.11.6561

81. Koch, B.E., Stougaard, J., and H.P. Spaink: Spatial and temporal expression patterns of chitinase genes in developing zebrafish embryos. *Gene Expr Patterns* 14, 69-77 (2014)
DOI: 10.1016/j.gep.2014.01.001

82. Koch, B.E, Stougaard, J, and H.P. Spaink: Keeping track of the growing number of biological functions of chitin and its interaction partners in biomedical research. *Glycobiology* 25, 469-482 (2015)
DOI: 10.1093/glycob/cwv005

83. Aerts, J.M., Hollak, C.E., van Breemen, M., Maas, M., Groener, J.E., and R.G. Boot: Identification and use of biomarkers in Gaucher disease and other lysosomal storage diseases. *Acta Paediatr Suppl* 94, 43-46 (2005)
DOI: 10.1080/08035320510028094

84. Grace, M.E., Balwani, M., Nazarenko, I., Prakash-Cheng, A., and R.J. Desnick: Type 1 Gaucher disease: null and hypomorphic novel chitotriosidase mutations-implications for diagnosis and therapeutic monitoring. *Hum Mutat* 28, 866-873 (2007)
DOI: 10.1002/humu.20524

85. Weigel, P.H., West, C.M., Zhao, P., Wells, L., Baggenstoss, B.A., and J.L.Washburn: Hyaluronan synthase assembles chitin oligomers with -GlcNAc(α1→)UDP at the

reducing end. *Glycobiology* 2, 632-643 (2015) DOI: 10.1093/glycob/cwv006

86. Weigel, P.H. Hyaluronan Synthase: The Mechanism of Initiation at the Reducing End and a Pendulum Model for Polysaccharide Translocation to the Cell Exterior.*Int J Cell Biol.* 2015; (2015) DOI: 10.1155/2015/367579

87. Turano, E., Busetto, G., Marconi, S., Guzzo, F., Farinazzo, A., Commisso, M., Bistaffa, E., Angiari, S., Musumeci, S., Sotgiu, S., and B Bonetti: Neurotoxicity and synaptic plasticity impairment of N-acetylglucosamine polymers: implications for Alzheimer's disease. *Neurobiol Aging* 36, 1780-1791 (2015) DOI: 10.1016/j.neurobiolaging.2014.12.033

88. Castellani, R.J., Perry, G., and M.A. Smith: The role of novel chitin-like polysaccharides in Alzheimer disease. *Neurotox Res* 12, 269-274 (2007) DOI: 10.1007/BF03033910

89. Castellani, R.J., Siedlak, S.L., Fortino, A.E., Perry, G., Ghetti, B., and M.A. Smith: Chitin-like polysaccharides in Alzheimer's disease brains. *Curr Alzheimer Res* 2, 419-423 (2005) DOI: 10.2174/156720505774330555

90. Sotgiu, S., Musumeci, S., Marconi, S., Gini B, and B. Bonetti: Different content of chitin-like polysaccharides in multiple sclerosis and Alzheimer's disease brains. *J Neuroimmunol* 197, 70-73 (2008) DOI: 10.1016/j.jneuroim.2008.03.021

91. Stern, R., Asari, A. R. and K.N. Sugahara: Size- specific fragments of hyaluronan: an information-rich system. *Eur. J. Cell Biol* 85, 699-715 (2006) DOI: 10.1016/j.ejcb.2006.05.009

92. Wattenberg, L.W., Patterson, S., and J.D. Antonides: Chitin or chitin-like glycans as targets for late-term cancer chemoprevention. *Cancer Prev Res* (Phila) 3, 1519-1522 (2010) DOI: 10.1158/1940-6207.CAPR-09-0230

93. Saiki, I., Murata, J., Nakajima, M., Tokura, S., and I. Azuma: Inhibition by sulfated chitin derivatives of invasion through extracellular matrix and enzymatic degradation by metastatic melanoma cells. *Cancer Res* 50, 3631-3637 (1990)

94. Murata, J, Saiki, I., Makabe, T., Tsuta, Y., Tokura, S., and I. Azuma: Inhibition of tumor- induced angiogenesis by sulfated chitin derivatives. *Cancer Res* 51, 22-26 (1991)

95. Benitez, A., Yates, T.J., Lopez, L.E., Cerwinka, W.H., Bakkar, A., and V.B. Lokeshwar: Targeting hyaluronidase for cancer therapy: antitumor activity of sulfated hyaluronic acid in prostate cancer cells. *Cancer Res* 71, 4085-4095 (2011) DOI: 10.1158/0008-5472.CAN-10-4610

96. Rosén, C., Andersson, C.H., Andreasson, U., Molinuevo, J.L., Bjerke, M., Rami. L, Lladó, A, Blennow, K., and H. Zetterberg: Increased Levels of Chitotriosidase and YKL-40 in Cerebrospinal Fluid from Patients with Alzheimer's Disease. *Dement Geriatr Cogn Dis Extr* 4, 297-304 (2014) DOI: 10.1159/000362164

97. Watabe-Rudolph, M., Song, Z., Lausser, L., Schnack, C., Begus-Nahrmann, Y., Scheithauer, M.O., Rettinger, G., Otto, M., Tumani, H., Thal, D.R., Attems, J., Jellinger, K.A., Kestler, H.A., von Arnim, C.A., and K.L. Rudolph: Chitinase enzyme activity in CSF is a powerful biomarker of Alzheimer disease. *Neurology* 78, 569-577 (2012) DOI: 10.1212/WNL.0b013e318247caa1

98. Choi, J., Lee, H.W., and K. Suk: Plasma level of chitinase 3-like 1 protein increases in patients with early Alzheimer's disease. *J Neurol* 258, 2181-2185 (2011) DOI: 10.1007/s00415-011-6087-9

99. Alonso, R., Pisa, D., Marina, A.I., Morato, E., Rábano, A., and L. Carrasco: Fungal infection in patients with Alzheimer's disease. *J Alzheimers Dis* 41, 301-311 (2014)

100. Pisa, D., Alonso, R., Rábano, A., Rodal, I., and L. Carrasco: Different Brain Regions are Infected with Fungi in Alzheimer's Disease. *Sci Rep.* 5, 15015 (2015) DOI: 10.1038/srep15015

101. Pisa, D., Alonso, R., Juarranz, A., Rábano, A., and L. Carrasco: Direct visualization of fungal infection in brains from patients with Alzheimer's disease. *J Alzheimers Dis* 43, 613-624 (2015)

102. Whelan, W.J.: The initiation of glycogen synthesis. *Bioessays* 5,136-140 (1986) DOI: 10.1002/bies.950050312

103. Nilsson, J., Halim, A., Larsson, E., Moslemi, A.R, Oldfors, A., Larson, G., and J. Nilsson: LC-MS/MS characterization of combined glycogenin-1 and glycogenin-2 enzymatic

activities reveals their self-glucosylation preferences. *Biochim Biophys Acta* 1844, 398-405 (2014)
DOI: 10.1016/j.bbapap.2013.11.002

104. Smythe, C., and P. Cohen: The discovery of glycogenin and the priming mechanism for glycogen biogenesis. *Eur J Biochem* 200, 625-631 (1991)
DOI: 10.1111/j.1432-1033.1991.tb16225.x

105. Alonso, M.D., Lomako, J., Lomako, W.M., and W.J. Whelan: A new look at the biogenesis of glycogen. *FASEB* 9, 1126-1137 (1995)

106. Moslemi, A.R., Lindberg, C., Nilsson, J., Tajsharghi, H., Andersson, B., and A. Oldfors: Glycogenin-1 deficiency and inactivated priming of glycogen synthesis. *N Engl J Med* 362, 1203-1210 (2010)
DOI: 10.1056/NEJMoa0900661

107. Malfatti, E, Nilsson, J., Hedberg-Oldfors, C., Hernandez-Lain, A., Michel F., Dominguez- Gonzalez, C., Viennet, G., Akman, H.O., Kornblum, C., Van den Bergh, P., Romero, N.B., Enge,l A.G., DiMauro, S., and A. Oldfors: A new muscle glycogen storage disease associated with glycogenin-1 deficiency. *Ann Neurol* 76, 891-898 (2014)
DOI: 10.1002/ana.24284

108. Lomako, J., Lomako, W.M., and W.J.Whelan: Glycogenin: the primer for mammalian and yeast glycogen synthesis. *Biochim Biophys Acta* 1673, 45-55 (2004)
DOI: 10.1016/j.bbagen.2004.03.017

Abbreviations: AMCase, acidic chitinase; BLAST, basic local alignment search tool; ChT, chitinase; chit1, chitinase-1; CD44, cluster of determination 44; CLP, chitinase-like proteins; ECM, extracellular matrix; ER, endoplasmic reticulum; GAG, glycosaminoglycan; GlcNAc, N-acetylglucosamine; UDP-GlcNAc, uridine diphosphate N-acetylglucosamine; HA, hyaluronan, hyaluronic acid; HAS, HA synthase; HYAL, hyaluronidase; Mya, millions of years ago; UDP, PAPs, 3'-phosphoadenosine 5'-phosphosulfate; uridine diphosphate; UTP, uridine triphosphate.

Key Words: Chitin, Chitinase, Chitin Synthase, Chitin Lectins, Hyaluronan, Hyaluronidase, Hyaluronan Synthase, Alzheimer's Disease, Gaucher's Disease

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