Fish & Shellfish Immunology xxx (2015) 1–13

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

# Structural, functional, and evolutionary aspects of galectins in aquatic mollusks: From a sweet tooth to the Trojan horse

G.R. Vasta [a, *], C. Feng [a], M.A. Bianchet [b], T.R. Bachvaroff [c], S. Tasumi [a, 1]

[a] Department of Microbiology and Immunology, University of Maryland School of Medicine, and Institute of Marine and Environmental Technology, Columbus Center, 701 East Pratt Street, Baltimore, MD 21202, USA
[b] Department of Neurology, and Department of Biophysics & Biophysical Chemistry, The Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205, USA
[c] University of Maryland Center for Environmental Science, and Institute of Marine and Environmental Technology, Columbus Center, 701 East Pratt Street, Baltimore, MD 21202, USA

ARTICLE INFO

*Article history:*
Received 8 April 2015
Received in revised form
30 April 2015
Accepted 4 May 2015
Available online xxx

*Keywords:*
Galectin
Phytoplankton
Parasite
Microbial recognition
Glycan ligands

ABSTRACT

Galectins constitute a conserved and widely distributed lectin family characterized by their binding affinity for β-galactosides and a unique binding site sequence motif in the carbohydrate recognition domain (CRD). In spite of their structural conservation, galectins display a remarkable functional diversity, by participating in developmental processes, cell adhesion and motility, regulation of immune homeostasis, and recognition of glycans on the surface of viruses, bacteria and protozoan parasites. In contrast with mammals, and other vertebrate and invertebrate taxa, the identification and characterization of *bona fide* galectins in aquatic mollusks has been relatively recent. Most of the studies have focused on the identification and domain organization of galectin-like transcripts or proteins in diverse tissues and cell types, including hemocytes, and their expression upon environmental or infectious challenge. Lectins from the eastern oyster *Crassostrea virginica*, however, have been characterized in their molecular, structural and functional aspects and some notable features have become apparent in the galectin repertoire of aquatic mollusks. These including less diversified galectin repertoires and different domain organizations relative to those observed in vertebrates, carbohydrate specificity for blood group oligosaccharides, and up regulation of galectin expression by infectious challenge, a feature that supports their proposed role(s) in innate immune responses. Although galectins from some aquatic mollusks have been shown to recognize microbial pathogens and parasites and promote their phagocytosis, they can also selectively bind to phytoplankton components, suggesting that they also participate in uptake and intracellular digestion of microalgae. In addition, the experimental evidence suggests that the protozoan parasite *Perkinsus marinus* has co-evolved with the oyster host to be selectively recognized by the oyster hemocyte galectins over algal food or bacterial pathogens, thereby subverting the oyster's innate immune/feeding recognition mechanisms to gain entry into the host cells.



## 1. Introduction

Cell surface glycans contain complex information that when decoded by specific carbohydrate-binding proteins, can determine the nature and outcome of interactions between cells, or cells and the extracellular matrix (ECM). Among these proteins, the galectins [1–3] constitute a structurally conserved family of animal lectins defined by their affinity for β-galactosides, and a characteristic sequence motif in the carbohydrate recognition domain (CRD) [4,5]. Galectins are widely distributed in eukaryotic taxa, and their early emergence in evolution has been revealed by the presence of a protein with the galectin fold in the protistan parasite *Toxoplasma gondii*, and galectin-like proteins in the fungus *Coprinopsis cinerea* and in the sponge *Geodia cydonium* [6–8]. Galectins are present in the cytosol but also can be translocated into the nucleus, and in spite of lacking a typical secretion signal peptide [9], they can be secreted into the extracellular compartment by direct translocation across the plasma membrane [10–14]. Once secreted, galectins can

* Corresponding author. Tel.: +1 (410) 234 8826; fax: +1 (410) 234 8896.
*E-mail address:* GVasta@som.umaryland.edu (G.R. Vasta).
[1] Present address: Fisheries laboratory, The University of Tokyo, 2971-4 Bentenjima, Maisaka-cho, Nish-ku, Hamamatsu, Shizuoka 431-0214, Japan.

http://dx.doi.org/10.1016/j.fsi.2015.05.012
1050-4648/

bind to endogenous carbohydrate ligands on the cell surface or the ECM, namely glycoproteins or other glycoconjugates that display non-reducing terminal β-galactosides or polylactosamine chains [15]. These include laminin and fibronectin, mucins, lysosome-associated membrane proteins, and numerous cell surface signaling glycans, such as integrins and Muc1 [16–19]. In addition, some galectins can also recognize exogenous ligands, such as glycans on the surface of microbial pathogens [20–27].

## 2. Structural and functional features of the galectin family

Based on their domain organization, mammalian galectins have been classified in three types: "proto", "chimera", and "tandem-repeat" (Fig. 1A) [9]. Proto type galectins contain one CRD per subunit, and are usually homodimers of non-covalently-linked subunits. The chimera type galectins have a C-terminal similar to the proto type and a non-CRD N-terminal domain rich in proline and glycine. Tandem-repeat galectins, in which two CRDs are joined by a linker peptide, are monomeric. Proto- and tandem-repeat types comprise several distinct galectin subtypes, which have been numbered following the order of their discovery [28], and so far, 15 have been described in mammals [9,15,28,29]. Gal1, 2, 5, 7, 10, 11, 13, 14, and 15 are examples of the proto type galectins, of which Gal5 is a monomer, whereas all others are homodimers. Gal3 is the only chimera type galectin, whereas Gal4, 6, 8, 9, and 12 are tandem-repeat type galectins. Among ectothermic vertebrates such as teleost fish and amphibians, the three major galectins types identified in mammals are mostly present, although the subtypes are less diversified [4,30–33].

In contrast, invertebrates and earlier taxa such as sponges, fungi, and protista express galectins with domain organizations that in most cases do not fit within any of the three major galectin types described in mammals. Furthermore, some galectin-like proteins such as the mammalian lens crystallin protein GRIFIN (galectin related inter-fiber protein) and the galectin-related protein GRP (previously HSPC159; hematopoietic stem cell precursor) lack carbohydrate-binding activity [34,35]. As the zebrafish GRIFIN orthologue is endowed with the typical carbohydrate binding activity of galectins, it has been proposed that the mammalian GRIFIN is a product of evolutionary co-option [35].

Although relatively conserved from a structural standpoint, galectins display a surprising functional diversification. The biological roles of selected members of the galectin family have been elucidated only in the past few years [36]. The available information is fragmentary, however, and among the numerous subtypes expressed in mammals, the roles of only a few galectins such as Gal1, Gal3, and Gal9, have been elucidated to a considerable extent [37].

### 2.1. Functions of galectins in early development

Since their discovery, galectins have been proposed to participate in embryogenesis and development. This has been based on their binding to endogenous "self" carbohydrate moieties, such as polylactosamine-containing glycans, abundant at the cell surface and the ECM. Chicken galectins have been proposed to participate in myoblast fusion, whereas murine Gal1 and Gal3 would have roles in notochord development, somitogenesis, and development of muscle tissue and central nervous system [11,28,32,38,39]. In recent years, the roles of galectins in cancer metastasis and angiogenesis have been investigated in detail, with promising potential for therapeutic intervention [40–42]. In addition, the availability of non-mammalian genetically tractable model organisms endowed with a less diversified galectin repertoire such as *Drosophila melanogaster*, *Caenorhabditis elegans*, and zebrafish (*Danio rerio*) have become attractive alternatives for investigating functional aspects of galectins from an evolutionary standpoint [43].

### 2.2. Functions of galectins in innate and adaptive immunity

By binding to endogenous carbohydrate ligands, mammalian galectins such as Gal1, Gal3 and Gal9, mediate diverse biological processes that are key to regulation of innate and adaptive immune homeostasis [44–49]. For example Gal1 participates in acute and allergic inflammation [29], and influences the ability of macrophages to control intracellular infections either by inhibiting microbicidal activity or inducing host-cell apoptosis [50]. With regards to adaptive immune functions, galectins and their ligands have been proposed as regulators of immune cell homeostasis [50].

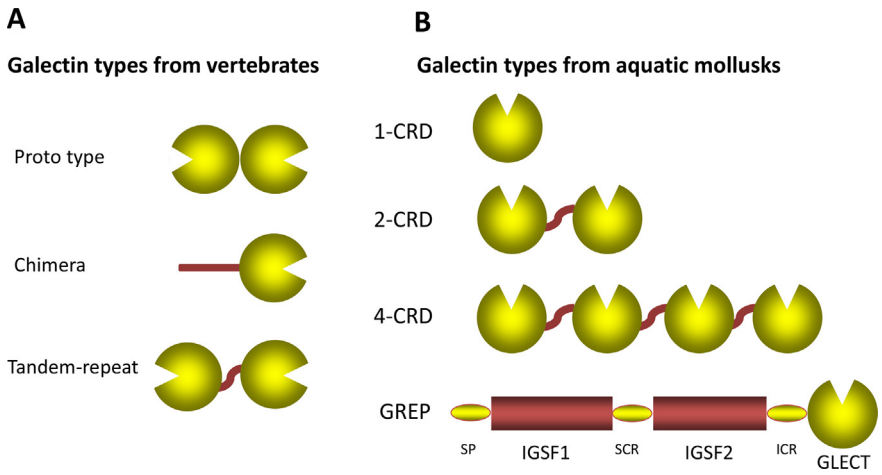


**Fig. 1. Domain organization of galectin types in vertebrates and aquatic mollusks. (A)** Schematic representation of the domain organization of the three galectin types (proto, chimera, and tandem repeat) described in vertebrate species. **(B)** Schematic illustration of the domain organization in the two most prevalent galectin types (2-CRD and 4-CRD) described in aquatic mollusk species, and the two types described in single reports [1-CRD from *C. gigas* (61) and GREP from *B. glabrata* (72)]. GREP is a chimeric protein in which a C-terminal galectin domain (GLECT) is joined via a short interceding region (ICR) to two immunoglobulin superfamily domains (IgSF1 and IgSF2) separated by a small connecting region (SCR), and with the signal peptide (SP). The sequence of the 1-CRD galectin from the Pacific oyster *C. gigas* (the only single CRD galectin reported so far in aquatic mollusks) appears to be a single domain of the 2-CRD galectin CgGal9 (EKC40501).

Please cite this article in press as: G.R. Vasta, et al., Structural, functional, and evolutionary aspects of galectins in aquatic mollusks: From a sweet tooth to the Trojan horse, Fish & Shellfish Immunology (2015), http://dx.doi.org/10.1016/j.fsi.2015.05.012