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REVIEW

Secreted immunoglobulin domain effector molecules of invertebrates and management of gut microbial ecology

Assunta Liberti[1] · Ojas Natarajan[2,4] · Celine Grace F. Atkinson[3,4] · Larry J. Dishaw[2,4]

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Abstract
The origins of a "pass-through" gut in early bilaterians facilitated the exploration of new habitats, motivated the innovation of feeding styles and behaviors, and helped drive the evolution of more complex organisms. The gastrointestinal tract has evolved to consist of a series of interwoven exchanges between nutrients, host immunity, and an often microbe-rich environmental interface. Not surprisingly, animals have expanded their immune repertoires to include soluble effectors that can be secreted into luminal spaces, e.g., in the gut, facilitating interactions with microbes in ways that influence their settlement dynamics, virulence, and their interaction with other microbes. The immunoglobulin (Ig) domain, which is also found in some non-immune molecules, is recognized as one of the most versatile recognition domains lying at the interface of innate and adaptive immunity; among vertebrates, secreted Igs are known to play crucial roles in the management of gut microbial communities. In this mini-review, we will focus on secreted immune effectors possessing Ig-like domains in invertebrates, such as the fibrinogen-related effector proteins first described in the gastropod *Biomphalaria glabrata*, the Down syndrome cellular adhesion molecule first described in the arthropod, *Drosophila melanogaster*, and the variable region-containing chitin-binding proteins of the protochordates. We will highlight our current understanding of their function and their potential role, if not yet recognized, in the establishment and maintenance of host-microbial interfaces and argue that these Igs are likely also essential to microbiome management.

Keywords Immunoglobulin domain · Innate immunity · Host-microorganism interaction · Secreted immune molecules · Invertebrate immunity · Microbiota

Introduction

The evolution of food capture and digestion first involved the origin of a gastric cavity, still present in the modern Cnidarians. While various derived and unique gut architectures have arisen during the diversification of bilaterians (e.g., see special issue; Hartenstein and Martinez 2019), the evolution of a "pass-through" or tubular gut giving rise to modern gastrointestinal (GI) systems represents an important innovation facilitating the evolution of more complex organisms (Fig. 1). For example, among the early metazoans, the evolution of muscles attached to rigid skeletal structures (not necessarily bones) and a pass-through gut forever changed how animals could explore the early seas (Holland 2015). The capacities of burrowing, exploring, and eating, while disposing of waste on the opposite side of their bodies, permitted the settlement of additional habitats and the diversification of feeding styles. The GI tract acquired more complex functionalities that included interactions with beneficial microbes, as well as encounters with pathogens. Indeed, it is well recognized that (i) diverse functions in the gut depend on microbial communities inhabiting it; (ii) host defense systems arose not only to protect tissue from pathogenic attack but also for supporting the growth of specific communities of mutualistic microorganisms;

✉ Assunta Liberti

✉ Larry J. Dishaw
ldishaw@usf.edu

1 Biology and Evolution of Marine Organisms (BEOM), Stazione Zoologica Anton Dohrn, Naples, Italy

2 Department of Pediatrics, Morsani College of Medicine, University of South Florida, Tampa, FL, USA

3 Department of Cell Biology, Microbiology, and Molecular Biology, University of South Florida, Tampa, FL, USA

4 Division of Molecular Genetics, Children's Research Institute, St. Petersburg, FL, USA

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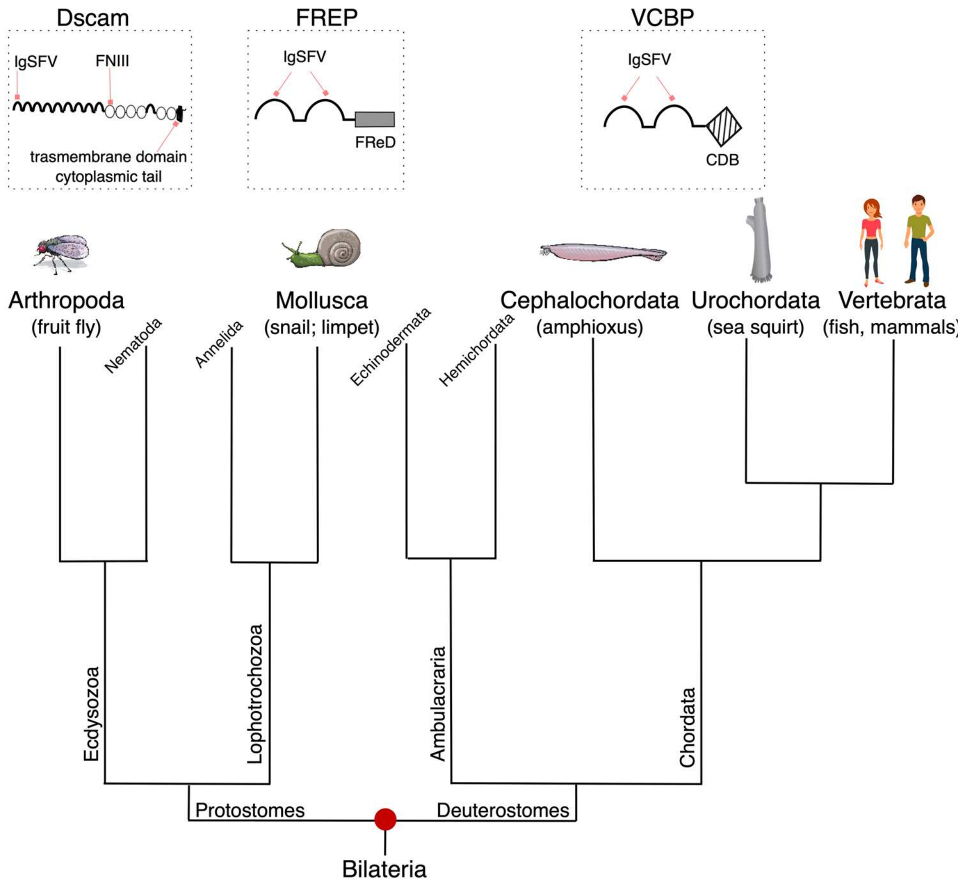


Fig. 1 Simplified phyletic distribution of metazoan IgSF-containing molecules. Schematic cladogram representation of major phyla following bilaterian divergence, emphasizing major clades where a "pass-through" tubular gut evolved. Phyla, such as Arthropoda, Mollusca, and Chordata, with IgSF-containing immune effectors discussed herein, are highlighted. Representatives of IgSF-containing molecules with immune-related functions have yet to be described in phyla represented by smaller characters. Protein structures of Dscam in arthropods, FREPs in gastropod molluscs and VCBPs in protochordates are reported. VIgSF, immunoglobulin superfamily variable domain; FNIII, fibronectin type III domain; FReD, fibrinogen-related domain; CDB, chitin-binding domain

and (iii) these types of interaction have been identified in most animal taxa, revealing a crosstalk with ancient origins (McFall-Ngai 2007; Round and Mazmanian 2009; Clemente et al. 2012; Dishaw et al. 2014).

It is important to reflect, however, on the complexity of the interplay: the host must maintain a balance between tolerating commensal microbial species while resisting those that are pathogenic, whereas the microbiota often manipulates the host immune repertoire (and shapes selective pressures) to best suit its survival. Hence, a more comprehensive understanding of the physical and genetic interactions between host immune defenses and the microbiome may help reveal the symbiotic underpinnings that helped shape the evolution of metazoans (Gilbert et al. 2012). The nature and patterns regulating domain organization among secreted immune effectors may also help reveal mechanistic insights for how symbiotic interactions interface with host immune recognition and responses. The diversification of mucosal immune responses, together with a more compartmentalized digestive tract to manage and digest the complexity of dietary antigens passing through the internal confines of animals, has been fundamental to bilaterian diversification (Fig. 2). One example was to innovate the release (e.g., secrete) of soluble immune effectors into the lumen of the GI tract. Here, these soluble molecules facilitate neutralization of antigens (including microbes with unknown intentions) and/or impact their ecological interactions before they settle and engage the epithelial surfaces; while sometimes cytotoxic, e.g., antimicrobial peptides, many of the soluble effectors instead bind microbial or antigenic surfaces and alter their intentions or capabilities. These neutralized antigens can more easily exit the animal while posing less of a threat; the bound mediators may also impact microbial settlement behaviors like the formation of biofilms. And because the antigenic surfaces are bound or tagged, gaining entry (past epithelial barriers) can also facilitate their recognition and elimination via phagocytosis, i.e., opsonization.

A large diversity of soluble immune molecules has evolved, some with unlimited recognition potential. While some, like many antimicrobial peptides, often lack recognized protein structural domains, other effector proteins include a limited set of protein domains that can be recognized by established receptors on immunocytes, facilitating recognition and proper downstream responses (Litman et al. 2005). Immune effector molecules can include a variety of recognition domains, such as immunoglobulin (Ig), leucin-rich repeat (LRR), and/or calcium-binding (C-type) lectin (CTL) domains; often, two or more unrelated domain types are coupled, for example, IgSF and lectin domain proteins have evolved in many animal taxa (Cannon et al. 2002, 2004; Dheilly et al. 2015; Gorbushin 2019). In this mini-review, we will focus on soluble immune effectors possessing Ig-like domains, since the Ig domain is recognized as one of the most versatile recognition domains (Williams and Barclay 1988; Cannon et al. 2010) and when secreted into the gut lumen has evolved to become essential to the management of gut microbial communities in the chordate lineage (Dishaw et al. 2014; Pabst and Slack 2020). It is our intention to cover essential concepts and discoveries; unfortunately, space limitations prevent an exhaustive overview and may result in the inadvertent omission of important findings.

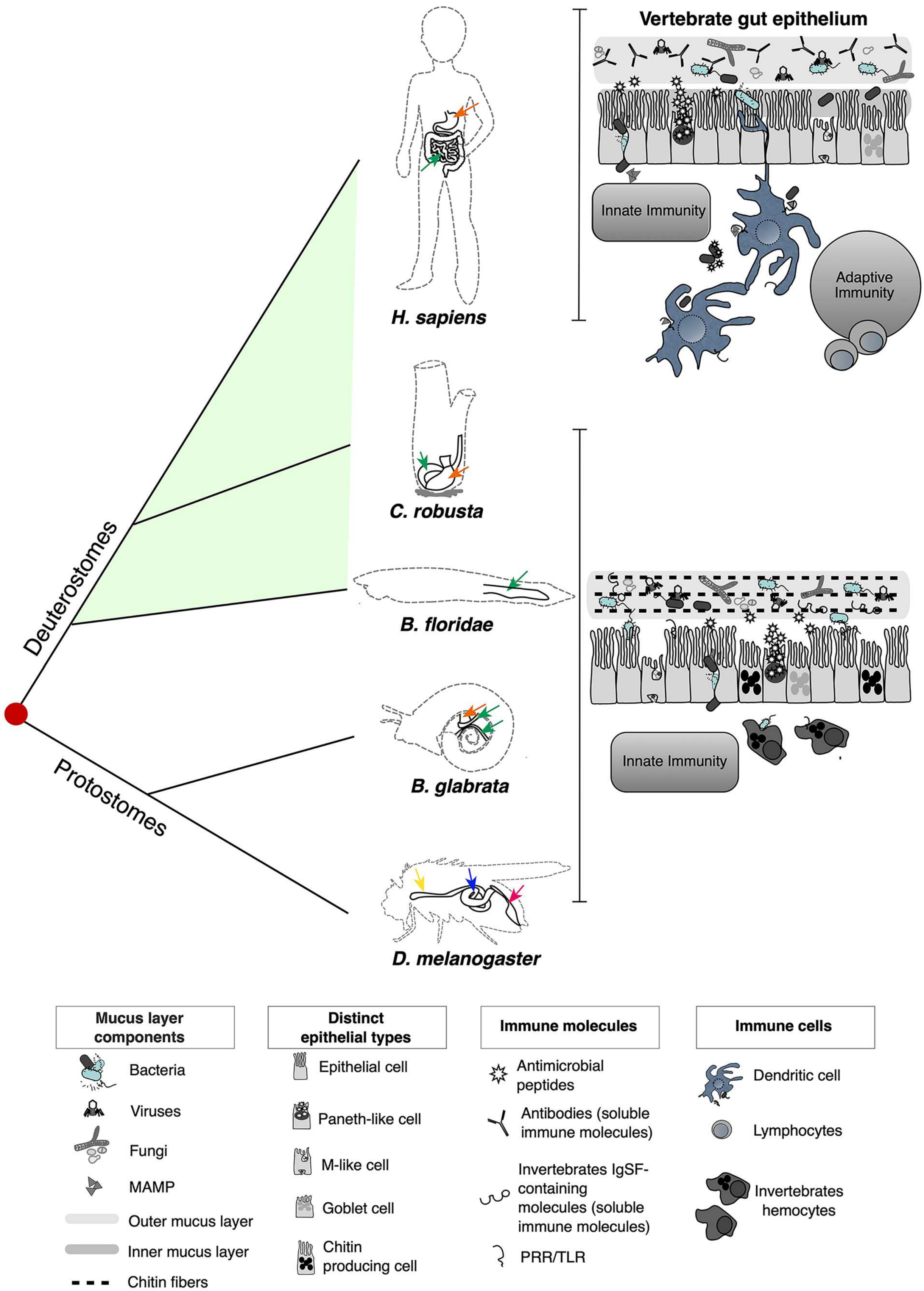
Immunoglobulin (Ig) domain in immune molecules

The Ig domain is a compact, globular structure of approximately 110 amino acids and typically contains an intrachain disulfide bond, while forming the antigen-binding domains of the classical antigen-binding receptors that include soluble immunoglobulins (or antibodies) and membrane-bound B cell and T cell antigen receptors (BCR and TCR) in the vertebrates. The name immunoglobulin results from studies to define the domain structure of antibodies, but it provides little information about the significance of this structure, contrary to other domains such as LRR and CTL. Beyond the forementioned vertebrate immune molecules, diverse proteins can possess Ig-type domains that extend recognition, binding and adhesion properties to a broad range of proteins with immunological as well as developmental functions (Sun et al. 1990; Su et al. 1998; Barclay 2003). By possessing Ig-like domains, these proteins belong to the immunoglobulin superfamily (IgSF) that includes adaptive and innate immune receptors as well as various accessory/adhesion molecules (Cannon 2009). A variety of biological features, including the ability to form homo- and heterodimers (Su et al. 1998; Barclay 2003), makes IgSF-like domains valuable to various aspects of recognition, e.g., self/non-self receptors, including Fc receptor classes found on immunocytes recognizing antibodies bound to antigens. Because of this, variations of this astonishingly versatile domain have now been identified in most extant taxa, with distantly related forms even recognized in bacteria (Bodelon et al. 2013) and viruses (Farre et al. 2017), where some DNA viruses have presumably captured or co-opted IgSF-domains (from animal genomes) to facilitate entry into host cells.

The IgSF domain has diversified over time into at least three major operational subclasses: variable (V), intermediate (I), and constant (C1 or C2), based on its size and other structural features; multiple domain types can be found in the same receptor molecules (Williams and Barclay 1988; Harpaz and Chothia 1994; Cannon 2009; Cannon et al. 2010). In the attempt to understand the phylogeny of immune receptor classes, researchers have focused on searching for and examining sequence orthologs of antibody classes and TCR in species outside the jawed vertebrates. The resulting sequences, when they exist, are often very divergent due to the polymorphic nature of the IgSF-domains across taxa. In vertebrates, different types of antibodies have evolved that are secreted into mucosal environments for immune protection, such as IgT of teleost fishes, IgX of amphibians, and IgA of mammals. Many studies have focused on investigating the role of IgA in the gut milieu, acting not just to protect against pathogens and toxins, but in shaping gut microbiota composition and modulating bacteria behaviors (Okai et al. 2017; Pabst and Slack 2020). In amphibians, IgX is a likely ortholog of IgA and is expressed in the gut and thought to be important to mucosal immunity (Mussmann et al. 1996). In fact, removal of the thymus early in development revealed a T cell independent immune response when exposed to intestinal microbiota, suggesting that IgX plays important roles in shaping gut microbial colonization (Mashoof et al. 2013). Among teleosts, and more distantly related to IgA but with some functional similarity with IgM (Piazzon et al. 2016), are the IgT mucosal antibody molecules, which are also expressed in the gut and coat most of the intestinal bacteria; in fact, depletion of IgT was found to induce dysbiosis with a marked overgrowth of pathobionts (or microbes with the capacity to become pathogenic) (Xu et al. 2020). Debating homology of these molecules is unnecessary given that common selective pressures have driven diverse taxa to innovate and repurpose various extracellular secreted immune molecules possessing Ig domains for the management of the complex antigenic nature of the gut. In this mini-review, we focus on several IgSF effectors with soluble and secreted forms found in invertebrates, with the aim to also discuss their potential role, if not yet recognized, in the establishment and maintenance of homeostasis in the gut.

Ig-Type immune molecules of invertebrates and their interaction with microbes

Invertebrates do not rely on adaptive immune responses, but on innate immune mediators. It has been recognized recently that some of these can mediate trained immunity, which can include a memory component (Gordy et al. 2015). Some effectors also possess diversified innate immune molecules with IgSF domains, and these include the fibrinogen related proteins (FREPs) first identified in the gastropod *Biomphalaria glabrata* (reviewed in (Adema 2015), the Down syndrome cellular adhesion molecule (Dscam) of arthropods (Watson et al. 2005; Dong et al. 2006), and the variable


Vertebrate gut epithelium
Innate Immunity
Adaptive Immunity
H. sapiens
C. robusta
B. floridae
B. glabrata
D. melanogaster
Deuterostomes
Protostomes
Innate Immunity
Mucus layer components
Bacteria
Viruses
Fungi
MAMP
Outer mucus layer
Inner mucus layer
Chitin fibers
Distinct epithelial types
Epithelial cell
Paneth-like cell
M-like cell
Goblet cell
Chitin producing cell
Immune molecules
Antimicrobial peptides
Antibodies (soluble immune molecules)
Invertebrates IgSF-containing molecules (soluble immune molecules)
PRR/TLR
Immune cells
Dendritic cell
Lymphocytes
Invertebrates hemocytes

◂**Fig. 2** Digestive tract compartmentalization and main features of vertebrate and invertebrate gut mucosal epithelium are represented (reprinted from (Dishaw et al. 2012; Liberti et al. 2021). The simplified cladogram (left side) reveals major clades and representatives discussed: the Protostomes, which include Arthropods, such as the fruit fly *Drosophila melanogaster* and the Molluscs, which include gastropods like *Biomphalaria glabrata*, and Deuterostomes, which include Chordates such as *Branchiostoma floridae, Ciona robusta* and, vertebrates like *Homo sapiens*. The digestive tract is highlighted in each, revealing distinct compartmentalization, indicated by different colored arrows (in each case, the anterior portion of the gut, not shown, includes an often complex and derived pharynx structure). In *D. melanogaster*, yellow arrow points to the foregut, whereas the midgut and the hindgut are blue and magenta arrows, respectively. In *B. glabrata*, *B. floridae*, *C. robusta*, and *H. sapiens*, the stomach and the intestine are indicated with orange and green arrows, respectively. For simplification, the human small and large intestines are labeled as "intestine." (right side) Illustration of gut mucosal immunity, emphasizing barrier defense strategies of invertebrates and vertebrates. In invertebrates, the gut epithelium can consist of distinct epithelial lineages that represent a primary barrier of defense, governed by innate immune phenomena characterized by the secretion of mucus (that often consists also of chitin fibers), antimicrobial peptides (AMPs), and soluble immune molecules such as immunoglobulin superfamily (IgSF) molecules. The mucus layers are often colonized by diverse microorganisms, including bacteria, viruses, and fungi. In the basolateral side, a distinct population of hemocytes, i.e., granular amoebocytes, resides in the laminar connective tissue and express diverse pattern recognition receptors (PRRs), also present on overlying epithelial cells, that can be used for sampling microbes. In vertebrates, barrier defenses of gut epithelium are also characterized by distinct epithelial lineages and include secretion of mucus (that organizes as a compact and firmly attached inner layer and a looser outer layer), AMPs, and other soluble immune molecules like antibodies. The secreted outer mucus layers are often colonized by diverse microorganisms, including bacteria, viruses, and fungi. On the basolateral surface of the epithelium, the innate immune response is coupled with the specialized adaptive immune system. Indeed, innate immune cells, like dendritic cells (DCs) that populate this area, sample luminal antigens via PRRs and present them to the adaptive immune system that includes gut-specific lymphocytes of both T and B cell lineages, thus triggering the maturation of immunity and the recruitment of additional cell types

region-containing chitin-binding proteins (VCBPs) found in protochordates, such as the cephalochordate *Branchiostoma floridae* and the urochordate *Ciona robusta* (Liberti et al. 2015). Although many immune-related IgSF-containing receptors undergo somatic rearrangement and diversification, others do not, and this is particularly true for IgSF effectors of invertebrates, which are mostly restricted to the accumulation of germ-line polymorphisms that accumulate in outbred populations (Litman et al. 2007; Wcisel and Yode 2016; Wcisel et al. 2017). Even in the absence of the necessary genetic machinery to generate recombination-based diversity (Azumi et al. 2003; Yuan et al. 2015; Flajni 2018), strong selection drives innovation. Accumulation of genetic polymorphisms that may enhance recognition potential in some IgSF-containing proteins suggests the necessity to recognize a large diversity of molecular targets that can include diversified pathogen receptors (Zhang et al. 2004).

FREPs of gastropods

The FREPs are soluble molecules first identified in Mollusca, both in Bivalves and in Gastropods; however, in the latter, they possess, in addition to the fibrinogen-related domain (FReD), one or two IgSF domains of the V-type subclass, thus representing a unique molecular architecture (Adema 2015; Gordy et al. 2015) (Fig. 1). The presence of IgSF domain(s) are observed only in gastropods FREPs, which are a subset of FReD proteins found in various animal taxa where they are characterized only by the fibrinogen-related domain. These proteins were first identified in the freshwater snail *B. glabrata* resistant to the infection of digenean trematodes, a group of parasites that almost exclusively use gastropods as obligatory intermediate hosts for their larval development. These parasites include the *Echinostoma paraensei* and *Schistosoma mansoni*, pathogens of rodent and human respectively (Monroy and Loker 1993; Zhang et al. 2004). Since *S. mansoni* is the etiological agent for the intestinal schistosomiasis, a debilitating infection disease that affects over 200 million people worldwide, the main investigations have remained focused and targeted on this host-parasite relationship to understand the biology and the transmission dynamics of the disease. As the only known example of somatic variation in *B. glabrata,* genome sequencing approaches identified as many as 14 FREP gene families; next-generation sequencing efforts later indicated that the number was likely greater (Dheilly et al. 2015; Lu et al. 2020), revealing variants that were products of gene conversion and/or point mutations in somatic cell lineages. These results suggested that some level of anticipatory-type immunity was at least partly responsible for long-term protection of repeated encounters among at least some invertebrate gene families (Zhang et al. 2004). The implication in these results was that FREPs were mainly expressed in circulating hemocytes that originate from precursor cells localized in amebocyte-producing organs, where somatic variation of these germline genes may occur during cell differentiation/expansion. It remains to be shown if single hemocytes can co-express multiple FREPs, and if the receptor repertoire of single hemocytes can be altered following unique or repeated immune challenges.

Studies based on transcriptome analyses of *B. glabrata* to investigate the diversity of FREPs transcripts led to the discovery of various proteins with both variable immunoglobulin (VIg) and lectin domains; as such, these were named variable immunoglobulin and lectin domain-containing molecules (VIgLs) (Dheilly et al. 2015). These proteins were subsequently found to be a large category of diverse lectins, with a modular domain structure comprising one or two IgSF domains and downstream lectin domains that included the CTL domain in the CTL-related proteins (CREPs) or the galectin domain in galectin-related protein (GREPs) (Dheilly et al. 2015). Further analysis of transcriptomes from another gastropod species, the snail *Littorina littorea*, revealed additional VIgLs, such as the C1q-related proteins (QREPs), the scavenger receptor cys-rich-related proteins (SREPs), *Zona pellucida*-related proteins (ZREPs), and *Helix pomatia* agglutinin (HPA) lectin-related proteins (HREPs), that each contain unique lectin domains coupled to IgSF (Gorbushin 2019). In gastropods, these VIgLs are proposed to function as non-self recognition molecules, and their presence/absence and location in the genome differ among the diverse gastropod species (Dheilly et al. 2015; Gorbushin 2019). Moreover, until now, these molecules have been identified and mostly investigated in gastropod blood cells (Dheilly et al. 2015; Wu et al. 2017; Gorbushin 2019), but their expression and role in the digestive tract remains unclear.

Arthropod FREPs, which for clarity are FReD proteins since they lack the IgSF domains, have been shown to both bind bacteria and mediate some interactions within the microbiome (Dong et al., 2009; Kulkarni et al. 2021) to help regulate and maintain gut immune-physiological homeostasis (Chauhan et al. 2020). While structurally distinct from gastropod FREPs, the function of FReDs in the gut suggests an important connection between these soluble immune effectors and the microbiota and may inspire further investigations for related roles among FREPs in the gut of gastropods. Moreover, besides arthropod FReDs that are secreted into the gut, lectin domains are also well recognized among pattern recognition receptors acting in gut immunity (Li et al. 2019; Zhang et al. 2021); these findings lend support for the possibility that in addition to pathogen-management in hemolymph by molluscan VIgLs, such as FREPs, these molecules may be contributing important functions in gut immunity and in the management of associated microbiomes.

DSCAMs of arthropods

A polymorphic homologue of Human Dscam was found exclusively in Arthropods (most recently reviewed in (Ng and Kurtz 2020), and specifically within Pancrustaceans, which include Crustaceans and Insects, such as the fruit fly, *Drosophila melanogaster* (Schmucker et al. 2000). The hypervariable (hv) Dscam gene generates diversity via alternative splicing and encodes surface (and to some extent, soluble) proteins that are members of the IgSF with a typical structure of ten IgSF domains, six fibronectin type III (FNIII) domains, a transmembrane domain, and a cytoplasmic tail (Fig. 1; Schmucker et al. 2000).

In *Drosophila* Dscam1, for example, an exceptionally complex genetic region encodes the resulting hv molecule, wherein three VIgSF domains are encoded by alternative exon clusters, with 20 constant exons and four variable exons (exon 4, 6, 9, and 17) encoding the resulting protein product. Specifically, the exon 4 cluster encodes 12 alternative copies, exon 6 encodes 48, exon 9 encodes 33, and exon 17 encodes 17. Via mutually exclusive splicing, only one exon from each cluster is used for the mature mRNA and can yield 19,008 possible combinations; when combined with either of two transmembrane domains, it results in an estimated 38,016 protein isoforms. Originally defined by its role in neuronal patterning, it was later shown that the hv Dscam was likely recruited into and being utilized in immune recognition pathways by hemocytes and other immune tissues (Watson et al. 2005; Dong et al. 2006), including the gut (Dong et al. 2009; Schwarz and Evans 2013). Because soluble versions were detected, first a proteolytic cleavage of membrane bound receptors (Watson et al. 2005) and then variants without transmembrane regions or cytoplasmic tails (Chou et al. 2009), it was hypothesized that it can also functions as an opsonin during phagocytosis of microbes.

Dscam has also been implicated in immune specificity and memory (Kurtz 2005; Sadd and Schmid-Hempel 2006), the mechanisms of which remain unknown but may be associated with trained immunity of responding somatic cell lineages (Netea et al. 2011; Chang et al. 2018). With the production of large assortment of diverse isoforms, this enhanced response can display specificity to challenging microbial targets (Dong et al. 2006; Hung et al. 2013). As many as seven paralogous hv Dscam genes have been described, evolving as orthologous forms in diverse arthropod lineages suggesting that these hypervariable recognition receptors have existed for hundreds of millions of years (Armitage et al. 2012). Due to variation in exon numbers, from more than 3300 to over 116,000 isoforms have been predicted to result from alternative splicing of hv Dscam orthologous gene transcripts across different taxa in a process largely driven by secondary structure of the pre-mRNA molecules (Xu et al. 2019). However, because upregulation of Dscam during infection or pathogen challenge is inconsistently reported among various arthropod taxa (Ng and Kurtz 2020), the complex immunological roles of these diverse transcript products remain unclear. The often taxon-specific and unique repertoire of these Dscam genes and their diverse transcriptional products suggests both recognition of diverse pathogens as well as a complex interplay with distinct microbial taxa that may shape selection of microbial communities in mucosal environments like the gut lumen.

We propose that this concept fits neatly within gut microbiome management; for example, Dscam, as well as the FREPs and other VIgL molecules, and the regulatory production of their diverse isoforms may be selectively linked to managing the normal flora of commensals and mutualists and that occasional encounters with pathogens selectively expand effectors that mediate neutralization and clearance.

It is likely that these effectors play a role in shaping the ecology of the gut environment by interacting with microbes and altering their settlement dynamics, virulence, or infection of host tissue. Whereas the products of FREP and the Dscam genes have been shown to be particularly effective against invading parasitic or bacterial pathogens, respectively, recent evidence does suggest a more expanded function that may be related to gut microbiota management. For example, in considering a role for FREPs in the gut, it remains possible that by regulating parasitic infections, they may be indirectly contributing to microbiome management since parasite burden can significantly impact the composition (structure) of the microbiome (Leung et al. 2018; Fredensborg et al. 2020). Furthermore, diverse functionality is not surprising given the size and multi-domain structure of these effector molecules, and it remains likely that they bind diverse targets and deliver multiple regulatory signals. An intriguing example is found in the simplest chordates (and discussed below), where another innovative secreted Ig effector molecule, the VCBPs, and their role in modulating the gut microbiota has been the primary focus of recent studies (Liberti et al. 2021).

VCBPs of protochordates

VCBPs are soluble immune effector molecules that are directly produced and secreted into the gut lumen by the stomach and intestinal epithelium; they are also produced by circulating hemocytes (Liberti et al. 2015). The VCBPs were first identified in amphioxus, *B. floridae*, and later in the sea squirt, *C. robusta* (Cannon et al. 2002, 2004; Liberti et al. 2015). The protein structure of these molecules is characterized, from the N- to the C- terminus, by a leader peptide, two tandem VIgSF domains and a chitin-binding domain (CBD) (Fig. 1). Briefly, in amphioxus, it has been observed that two of the five VCBP loci demonstrate an exceptional haplotypic and allelic diversity in the first VIgSF domain, generated by indel polymorphism (Dishaw et al. 2008, 2010; Liberti et al. 2015). From an evolutionary perspective, the VCBP 2 and 5 genes are polymorphic paralogous genes of amphioxus and worthy of additional consideration since they appear to lie at the interface between unique pathogen-driven selective pressures and host immunity. In addition, structural analyses revealed an unexpected head-to-tail orientation of the two VIgSF domains, which is in contrast to the head-to-head orientation observed in jawed vertebrate Igs and TCRs antigen-binding regions that result from dimerization of heavy and light chains (Hernandez Prada et al. 2006; Liberti et al. 2015). While it remains unclear why this unusual structure exists, it may have occurred as a result of single chain instabilities or the existence of a unique antigen-binding mechanism or even of other special requirements to achieving structural stability of the antigen binding region in VCBPs (Hernandez Prada et al. 2006). And while the gene sequences of the four identified VCBP molecules (namely *Cr*VCBP- A to -D) in *Ciona* show single nucleotide and indel polymorphisms, they lack the high degree of haplotypic variation observed in *BfVCBP 2* or *5* (Dishaw et al. 2011). The evolution of VCBP genes may help reveal specific microbial drivers of diversification in secreted effectors of the gut.

The function of these proteins in gut immunity has been most thoroughly investigated in *Ciona*, where VCBP-C has been shown to be involved in both immune defense and in the interaction with gut-derived microorganisms (Dishaw et al. 2011, 2016; Liberti et al. 2018, 2019). These immune effectors are expressed in immune competent tissues of *C. robusta*, such as blood tissue, and hemocytes, where they are secreted into the hemolymph and act as an opsonin, binding bacteria and increasing phagocytic activity (Dishaw et al. 2011). They are also expressed in the digestive tract, where they are first expressed and secreted by the epithelium during metamorphic development, revealing unique patterns of expression along the anterior–posterior axis and among diverse cell types (Liberti et al. 2014, 2015). As secreted effectors, the VCBPs are found within the gut lumen and also tethered within the mucus layers of the digestive tract (Dishaw et al. 2016), where they likely bind and interact with microorganisms (Fig. 3a). Thus, the VCBPs are likely acting as molecules that can influence the microbial ecology of the gut, and this includes shaping settlement dynamics, biofilms, and/or interactions among microbes.

Distinct binding characteristics have been observed for the two types of domains present in VCBP proteins. The VIgSF domains are able to bind various bacterial strains isolated from *Ciona* gut and affect, in vitro, their settlement and biofilm formation (Fig. 3b, c), whereas the CBD is able to interact with and become tethered to the chitin-rich mucus that coats the epithelium (Dishaw et al. 2016). In addition, the CBD has also been shown to recognize and bind chitin molecules present on the cell wall, sporangia, and spores of diverse fungal species isolated from *Ciona* gut (Fig. 3d, e; Liberti et al. 2019), suggesting that unbound VCBP can play a role in transkingdom interactions. While VCBP-C can bind fungal chitin, it remains unclear what this means for the host or fungal interactions with bacteria in the gut of *Ciona*. In contrast, the binding of the VIgSF domains to bacterial surfaces appears to impact behaviors and phenotypes (i.e., biofilm formation (Dishaw et al. 2016)), although the mechanisms remain unclear as do the specific cell surface targets involved. What remains interesting, and still a feature unique to VCBPs among secreted immune factors, is the ability to bind both bacteria and fungi on opposing ends of the same molecule to potentially influence important transkingdom interactions (e.g., Peleg et al. 2010; Deveau et al. 2018). Thus, how these effectors influence the biology and ecology of polymicrobial communities may help better inform

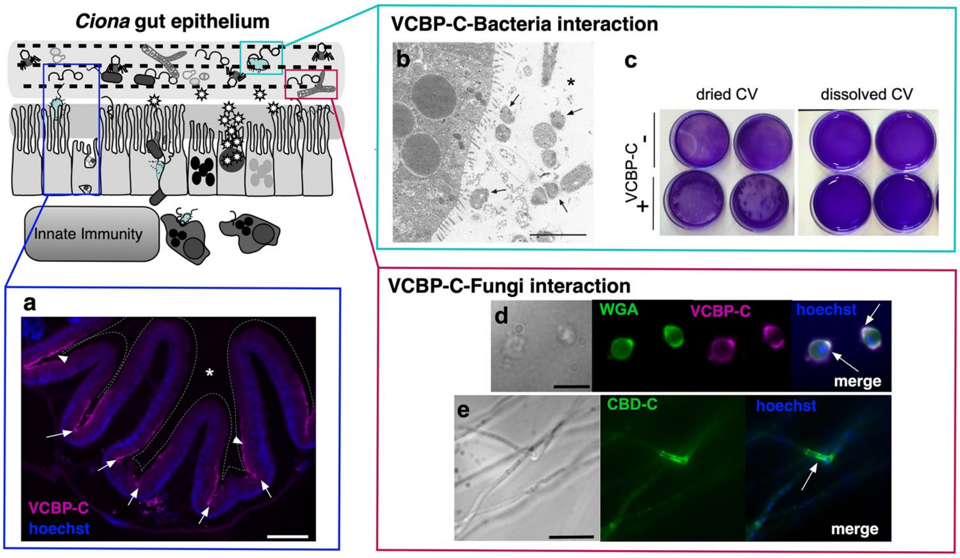


Fig. 3 *Ciona* VCBP-C gut localization and interactions with microorganisms. In *Ciona* gut epithelium, VCBP-C molecules are produced and secreted into the gut lumen by the epithelial cells of the digestive tract and can interact with diverse components of microbiota, such as bacteria and fungi. **a** Sections of stomach epithelium showing localization of VCBP-C (magenta) in both granules of cells localized in the crypts (arrows) and in the mucus lining the epithelium (arrowhead). **b** Immunogold staining, using specific anti-VCBP-C antibody, reveals VCBP-C bound to experimentally introduced bacteria, such as *Bacillus cereus*, localized both in the lumen and adjacent to stomach epithelium wall (arrows) (reprinted from (Dishaw et al. 2011). **c** Biofilm assay of *Shewanella* sp. grown for 4–5 days in the presence/absence of recombinant VCBP-C protein and visualized by crystal violet (CV) staining. Plates are shown with dried (left image) or solubilized/dissolved stain (in acetic acid; right image) that can be used for crude quantification of biofilm abundance, with a microplate reader at OD_{560} (reprinted from (Liberti et al. 2021). **d** Immunofluorescence with specific anti-VCBP-C antibody on fungal spores isolated from liquid culture of *Penicillium* sp. and incubated with recombinant VCBP-C protein shows binding of VCBP-C (magenta) to chitin molecules localized in specific regions of spore surface (e.g., bud scars, arrows). The specific binding of VCBP-C to chitin molecule is confirmed by co-localization with wheat germ agglutinin (WGA) staining (green), a lectin known to recognize chitin on fungal surfaces (reprinted from (Liberti et al. 2019). **e** Immunofluorescence with recombinant IgG1-Fc-chitin binding domain (CBD) of VCBP-C (IgG1-Fc-CBD-C) probe on whole *Penicillium* sp. fungi grown in liquid medium reveals binding of the CBD-C (green) probe to chitin molecules localized in specific regions of the fungal hyphae (arrow) (reprinted from (Liberti et al. 2019). Asterisk, gut lumen. White dotted lines highlight the surface morphology of the epithelium. Scale bar: **a** 100 µm; **b** 2 µm; **d** 10 µm; **e** 25 µm

our understanding of their influence on gut homeostasis and host physiology in humans (e.g., van Tilburg Bernardes et al. 2020; Boutin et al. 2021).

Conclusion

The evolution of a "pass-though" tubular gut facilitated the evolution of more complex organisms and the establishment of disparate mutualistic interactions with diverse microbiota. The awareness of the importance of the gut flora in the physiology and health of animals has opened the frontiers of investigations into the bi-directional interaction between the microbial world and the host immune system and reshaping the once firm belief that immune systems only evolved to engage with and defend against everything that is non-self (McFall-Ngai 2007). Investigating known IgSF-domain containing immune molecules, as discussed here, or searching for novel forms or re-focusing on the many other IgSF molecules identified in invertebrates with unknown functions (Vogel et al. 2003) while considering this "new" concept will facilitate a more comprehensive understanding of the diverse mechanisms underpinning the ecological maintenance of homeostasis within the digestive tract of animals. These data are also revealing secretory IgSF effector proteins as innovative milestones in the evolution of animal strategies to meet the sophisticated needs of the dynamic interplay between animals and their environment.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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