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The role of fibrinogen-related proteins in the gastropod immune response

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ABSTRACT

Fibrinogen-related proteins or FREPs constitute a large family of molecules, defined by the presence of a fibrinogen-related domain (FReD). These molecules are found in all animals and are diverse in both form and function. Here, we review the current understanding of gastropod FREPs, which are characterized by the presence of a fibrinogen domain connected to one or two immunoglobulin superfamily domains by way of a short interceding region. We present a historical perspective on the discovery of FREPs in gastropods followed by a summary of advances made in the nearly two decades of research focused on the characterization of FREPs in *Biomphalaria glabrata* (BgFREPs). Topics covered include BgFREP genomic architecture, predicted structure and known functions, structural comparisons between BgFREPs, and evidence of somatic diversification. Also examined are the expression patterns of BgFREPs during snail development and immunological challenges. Recent functional characterization of the role BgFREPs play in the defence response against digenean trematodes is also presented, as well as new data investigating the nucleotide-level genomic conservation of FREPs among Pulmonate gastropods. Finally, we identify areas in need of further research. These include confirming and identifying the specific binding targets of BgFREPs and elucidating how they later engage snail haemocytes to elicit an immunological response, precise mechanisms and importance of BgFREP diversification, characterizing the tissue expression patterns of BgFREPs, as well as addressing whether gastropod FREPs retain immunological importance in alternative snail-trematode associations or more broadly in snail-pathogen interactions.

1. Introduction

Immune factors that possess a fibrinogen domain (FBG) have emerged as being important components of the innate immunological response of invertebrates (reviewed in Hanington and Zhang, 2011 [1]). Often considered an essential aspect of the coagulation cascades of vertebrates, evidence from invertebrate animal models supports an equally important role for fibrinogen-related domains (FReDs) as stand-alone, or as domains of, innate immune factors. Fibrinogen domains are conserved throughout animal evolution. They are present in single-celled eukaryotes, such as the choanoflagellate *Monosiga brevicollis* [2], and found in great abundance in chordate genomes, as exemplified in the amphioxus *Branchiostoma floridae* [3]. Arthropod and mollusc model systems have helped to understand the functional roles that fibrinogen-related proteins (FREPs) play in invertebrate defence against pathogen challenge. The FREPs of these two phyla differ in their structural composition, but are unified by the presence of well-defined fibrinogen domains.

Within the clade Mollusca, FREPs have been identified in Bivalvia (several species of *Mytilus* mussels [4] [5], *Argopecten irradians* (Bay Scallop) [6], and *Crassostrea gigas* (Pacific Oyster) [7]) and Gastropoda (*Biomphalaria glabrata, Helisoma trivolvis*, *Bulinus truncatus*, *Lymnaea stagnalis*, *Helix aspersa* [8], *Biomphalaria pfeifferi* [9], *Aplysia californica* [10], *Littorina littorea* [11] and *Limax flavus* (Yellow Slug) (sialic-acid-binding lectins with a fibrinogen domain) [12]). The FREPs of gastropod molluscs are particularly interesting because of their unique structure, capacity for somatic diversification, and function in immune defense. While the FREPs described from organisms within Bivalvia thus far contain only a fibrinogen domain, gastropods possess a unique subset of FREPs that are the

Abbreviations: FREP, fibrinogen-related protein; FReD, fibrinogen-related domain; FBG, fibrinogen (domain); IgSF, immunoglobulin superfamily (domain).

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only known FREPs to combine immunoglobulin superfamily domains (IgSF) with their fibrinogen domain (excluding the sialic-acid-binding lectins of *L. flavus*). This particular type of FREP, sometimes referred to as IgSF-FREPs, are hypothesized to be an "evolutionary novelty" of Heterobranch molluscs [4,10]. These IgSF-containing FREPs play a central role in defence against digenean trematodes [13,14], a group of parasites that almost exclusively utilize gastropods as host for their larval development [15].

Gastropods are capable of mounting specific, cellular, and humoral immune responses following pathogen challenge, and evidence suggests they are able to generate more effective and specific secondary responses following homologous pathogen challenge [16–18]. Currently, among the most well characterized gastropod immune factors are the FREPs, which are part of a large gene family of polymorphic lectin-like molecules.

This review will focus on our current understanding of IgSF-containing FREPs as a unique group of immune molecules in gastropods (for a review on other gastropod immune mechanisms, please see that of Coustau et al. in this special issue). We will discuss the history of their discovery, how their structure may impact their functional roles in gastropod immune responses, and highlight conserved and unique features gastropod FREPs possess. We will also explore the known mechanistic basis of gastropod FREP diversification, and the potential role that these factors play in defending snails against parasitic flatworms.

1.1. Defining a FREP

Fibrinogen-related protein or FREP is a term often used to describe a protein containing a fibrinogen-related domain (FReD). As a descriptive term, FREP serves as a catchall that identifies a large group of molecules that are found in all animals, and are diverse in both form and function. While FREP remains an accurate term for encompassing FReD-containing proteins, it is not useful for differentiating one from another based on function or structure. The continued use of 'FREP' to identify any proteins containing a FReD has led to confusion when comparing FREPs between animals. For the purposes of this review, the term BgFREP will be used to describe FREPs characterized in the freshwater gastropod *B. glabrata,* which contain a fibrinogen domain connected to an immunoglobulin superfamily domain. It is from the *B. glabrata* model that we derive most of our understanding of gastropod FREPs, and we draw much of the information for this review from studies using this model organism. All other FREPs will be referred to using the species acronym as a prefix to 'FREP' to differentiate the various FREP structures and functions associated with specific studies.

1.2. Gastropods and digenean trematodes

Digenean trematodes rely upon molluscs, primarily gastropods, to complete their larval development. Having parasitized snails for nearly 200 million years, many species of trematode have formed obligate relationships with specific snail species, resulting in a host-parasite association often defined by high host specificity [15]. Differences in compatibility between snail and digenean trematode species have been well documented, with clear contrast in the ability of incompatible snails to successfully defend against challenge by specific trematode species compared to those snails that become infected [19,20].

When a trematode infects a snail, an intimate relationship is formed that balances host longevity and parasite reproduction. Trematodes have developed mechanisms to evade and even suppress the specific snail immune processes in order to enter, establish and reproduce within the snail host. However, not every trematode is able to infect each snail species equally, suggesting that snails have developed mechanisms to combat trematode infection. The primary determinant of host-parasite specificity we see in many snail-trematode examples could be the result of a phenotypic mismatch between these competitive factors, a hypothesis referred to as the match-mismatch hypothesis [21–23]. Alternatively, we cannot discount the complexity of the gastropod innate immune response and the evidence supporting a role for genotypic differences among snail populations that are known to impact compatibility. One component of the snail immune response is BgFREPs, soluble lectin-like factors that are known to interact with molecules secreted by and on the tegumental surface of trematodes, and have been demonstrated to be important in defining resistance to infection. BgFREPs may represent one of the mechanisms that have evolved in snails to counteract and defend against trematode infection. As more studies investigate this host–parasite relationship, support for this hypothesis continues to accumulate.

Existing knowledge of gastropod immunobiology is heavily influenced by studies examining the relationship between the snail *B. glabrata* and the digenean trematode *Schistosoma mansoni*. This imbalance in the literature is primarily due to the fact that *S. mansoni* is the etiological agent for the disease Schistosomiasis, which affects over 200 million people worldwide [24]. *B. glabrata* and closely related African and South American *Biomphalaria* species serve as the natural intermediate hosts for *S. mansoni. Biomphalaria* snails and *S. mansoni* are important laboratory models that have been extensively studied to further our understanding of the biology and transmission dynamics of schistosomiasis. Future long-term and sustainable efforts to disrupt transmission of schistosomes at the snail-stage of their life cycle are heavily reliant on improving our understanding of the factors that dictate compatibility between *Biomphalaria* snails and schistosomes.

Echinostomes have also been an instrumental infection model for studying the *Biomphalaria* immune response to trematodes. They have been and continue to be valuable for understanding trematode-mediated immunosuppression of the snail host and the underlying molecular processes. This immunosuppressive process has been key to furthering our understanding of compatibility between digenean trematodes and their molluscan hosts [25].

2. The discovery of FREPs in gastropods

It has been known since the 1960's that agglutinating molecules are present in the plasma of molluscs. In oysters, these lectin-like molecules possess opsonic properties that correlate with increased phagocytosis of rabbit erythrocytes by haemocyte monolayers when the red cells are incubated in lectin-containing plasma derived from *Crassostrea virginica* [26]. Later, in the 1970's and 80's, similar agglutinins were found in several other molluscs, including the gastropods, *Helix pomatia* [27] and *B. glabrata* [28]. These lectin-like factors were demonstrated to be involved in the recognition of non-self by binding to carbohydrate targets on haemocyte membranes of other species and pathogen-associated surfaces [29].

Gastropod agglutinating factors are known to be important for bacterial clearance [27], but more intriguing is their apparent role in the defence response to digenean trematode challenge. In the mid-1980's, several experiments revealed that an agglutination response occurs when trematode sporocysts are placed in snail plasma [30–33]. Specifically, the plasma of *B. glabrata* snail strains, resistant to the trematode *S. mansoni*, agglutinated aldehyde-fixed sporocysts, while susceptible strains did not [31]. Several subsequent experiments revealed that there were particular polypeptides present in plasma of resistant *B. glabrata* strains that

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