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Comparative immunogenomics of molluscs

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ABSTRACT

Comparative immunology, studying both vertebrates and invertebrates, provided the earliest descriptions of phagocytosis as a general immune mechanism. However, the large scale of animal diversity challenges all-inclusive investigations and the field of immunology has developed by mostly emphasizing study of a few vertebrate species. In addressing the lack of comprehensive understanding of animal immunity, especially that of invertebrates, comparative immunology helps toward management of invertebrates that are food sources, agricultural pests, pathogens, or transmit diseases, and helps interpret the evolution of animal immunity. Initial studies showed that the Mollusca (second largest animal phylum), and invertebrates in general, possess innate defenses but lack the lymphocytic immune system that characterizes vertebrate immunology. Recognizing the reality of both common and taxon-specific immune features, and applying up-to-date cell and molecular research capabilities, in-depth studies of a select number of bivalve and gastropod species continue to reveal novel aspects of molluscan immunity. The genomics era heralded a new stage of comparative immunology; large-scale efforts yielded an initial set of full molluscan genome sequences that is available for analyses of full complements of immune genes and regulatory sequences. Next-generation sequencing (NGS), due to lower cost and effort required, allows individual researchers to generate large sequence datasets for growing numbers of molluscs. RNAseq provides expression profiles that enable discovery of immune genes and genome sequences reveal distribution and diversity of immune factors across molluscan phylogeny. Although computational *de novo* sequence assembly will benefit from continued development and automated annotation may require some experimental validation, NGS is a powerful tool for comparative immunology, especially increasing coverage of the extensive molluscan diversity. To date, immunogenomics revealed new levels of complexity of molluscan defense by indicating sequence heterogeneity in individual snails and bivalves, and members of expanded immune gene families are expressed differentially to generate pathogen-specific defense responses.

1. Introduction

Historical observations of the association between exposure to disease and subsequent protection from future (human) illness eventually led to development of the smallpox vaccine by Jenner in the late 1700s (Owen et al., 2013), and of the Germ Theory, linking pathogens and disease (Walker et al., 2006). Studies of how immunity against pathogens is achieved culminated in our current understanding of immunology, mostly as it reflects the immune function of vertebrate animals. Some of the early studies of immunity, however, also benefitted from use of invertebrate organisms. Most famously, Metchnikoff discovered phagocytes and their role in immunity in starfish larvae (Metchnikoff, 1905). By using one (invertebrate) organism to make predictions of immune function in other (vertebrate) animals Metchnikoff gave rise to a new field of biology: comparative immunology. The power of comparative immunology begotten by investigating invertebrates is evident from landmark characterization of e.g. lectins (Prokop et al., 1968), antimicrobial peptides (Boman and Hultmark, 1987), Toll-like receptors (Lemaitre et al., 1996; Medzhitov et al., 1997), and RNA interference (Fire et al., 1998) that have expanded our understanding of immunity and revealed shared features among animals across phylogeny and evolution.

The study of immune function of molluscs (including snails, bivalves, cephalopods, others, see Fig. 1) is motivated importantly by notions that many molluscs are economically valuable food sources, especially in aquaculture (Carnegie et al., 2016), or may transmit infectious diseases of medical and veterinary relevance

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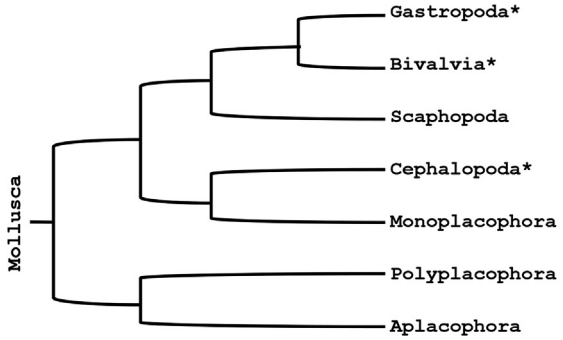

	ESTs					SRA	
	1990	2000	2010	2016	# species	2016	# species
Gastropoda*	0	1232	630146	647770	23	1985	182
Bivalvia*	0	0	347393	382612	37	2829	127
Scaphopoda	0	0	0	0	-	7	6
Cephalopoda*	0	0	46081	114034	6	225	38
Monoplacophora	0	0	0	0	-	2	1
Polyplacophora	0	0	498	1548	2	5	5
Aplacophora	0	0	0	1907	2	13	11

Fig. 1. Simplified phylogeny of the classes in the phylum Mollusca adapted from Kocot et al., 2011 and Smith et al., 2011. Asterisks indicate genome availability in public databases. For each class the number and increments of expressed sequence tag (EST) and short read archive (SRA) entries are indicated over time. Note that SRA entries were not available before 2008, coinciding with the advent of next-generation capabilities. Data were obtained by querying the SRA and EST databases of GenBank using name of class and modification dates as search terms.

(Adema et al., 2012). Moreover, the highly diverse phylum Mollusca is second in size among animals only to Arthropoda and represents the generally understudied lophotrochozoan protostomes, one of three lineages of metazoan animals, along with ecdysozoan protostomes and deuterostomes (e.g. Erwin et al., 2011). As such, study of molluscs will continue to broaden understanding of the evolution of immune function across the range of metazoan phylogeny, especially because recent insights suggest that molluscs are capable of sophisticated and specific immune responses (Adema and Loker, 2015; Coustau et al., 2015; Guo et al., 2015).

This review briefly discusses the view of molluscan immune capabilities as it developed from investigations before the availability of immunogenomics. We then present the more specific characterization of molluscan immunity that was afforded by studies benefitting from PCR and Sanger sequencing. Following is a discussion of molluscan immune capabilities discovered from genome mining and transcriptome analyses, especially by relatively easily-applied large-scale next-generation sequencing techniques. Lastly, we consider current limitations of utilizing NGS data and discuss the future of molluscan comparative immunology now that large sequence datasets are increasingly available.

2. Molluscan immunity

Historically, molluscan immunology has been studied in a small number of species represented within the diversity of the phylum Mollusca. Practical considerations that included ease of collection, animal size, reliable animal husbandry, selective rearing of genetic lineages, as well as relevance for disease transmission or economical (aquaculture) importance have focused consistent study toward a few species of the Gastropoda, and increasingly so in recent times to some representatives of the classes Bivalvia and Cephalopoda, to the exclusion of other molluscan classes (Fig. 1). Experimental approaches for initial immunological studies included the monitoring of responses of bivalve and gastropod molluscs following exposure to inorganic material (e.g. Indian ink; Tripp and Kent, 1967), to pathogens, introduced either by bacterial injection or through infection with parasites, notably digenean flatworms like *Schistosoma mansoni* that causes significant infectious disease when transmitted to humans (Tebeje et al., 2016). Snails were observed to rapidly clear bacteria from circulation and survive the exposure, with indications of elevated immunity, a more rapid clearance, after an initial encounter (Bayne, 1980; van der Knaap et al., 1983a, 1981). Some individual snails among populations of otherwise parasite-susceptible *Biomphalaria glabrata* proved naturally resistant to digenetic trematodes, with more rapid responses toward a secondary exposure (Lie and Heyneman, 1979). Susceptibility to parasite infection was determined by the genetic background of snail and parasites (Richards et al., 1992). Professional phagocytic cells termed hemocytes, dwelling in the tissues or circulating with the blood fluid of gastropods and bivalves, phagocytose or encapsulate pathogens, eliminating these with cell-mediated cytotoxicity involving lysosomal enzymes and production of reactive oxygen species (Adema et al., 1991; Granath and Yoshino, 1983; La Peyre et al., 1995; McKerrow et al., 1985; Mohandas et al., 1985; van der Knaap and Loker, 1990). Depending on the species, molluscs may have either a single type or several functionally different categories of hemocytes, and these cells may originate from connective tissue or specialized organs, termed the amoebocyte producing organ (APO) in gastropods (Jeong et al., 1983), or from the white body organ in cephalopods (Claes, 1996; Cowden, 1972). Recognition of nonself and subsequent immune activation is mediated through lectins, initially referred to as agglutinins or cytophilic receptors for foreignness, present as humoral factors or on the surface of hemocytes (Cheng et al., 1984; Michelson and Dubois, 1977; Mullainadhan and Renwrantz, 1986; Renwrantz and Cheng, 1977; Rögener et al., 1985; van der Knaap et al., 1983b). Lectins are non-enzymatic, non-antibody proteins that function as pattern recognition receptors (PRRs) by binding to repetitive carbohydrate surface determinants that characterize groups of pathogens (pathogen associated molecular patterns, PAMPs) such as lipopolysaccharide (LPS) and peptidoglycans of bacteria (Vasta and Ahmed, 2009) and activate immune responses. Contrary to expectations regarding animal immunity drawn from a vertebrate perspective of immune function, and by the observation of some level of immunological memory in gastropods (Lie and Heyneman, 1979), no indications were found in molluscs, or invertebrates in general, of lymphocytic defenses, i.e. no T-cells, B-cells or the rearranging genes that drive generation of antigen-specific receptors (Warr, 1981). As a consequence, invertebrates were deemed to possess a rather unsophisticated innate-type immunity, with a reliance only on invariable, germline-encoded genes for general broad immune recognition of categories of pathogenic organisms. However, Klein (1989) championed the importance of investigating the immunity of invertebrates from new perspectives that are not myopically biased by norms of vertebrate immunology.