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1 **Title: Towards a mechanism for poly(I:C) antiviral priming in oysters**

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7

8 **Abstract**

9 Viral diseases cause significant losses in aquacultures. Prophylactic measures, such as immune
10 priming, are promising control strategies. Treatment of the Pacific oyster (*Crassostrea gigas*)
11 with the double stranded RNA analog polyinosinic:polycytidylic acid (poly I:C) confers long
12 term protection against infection with Ostreid Herpesvirus 1, the causative agent of Pacific
13 oyster mortality syndrome. In a recent issue of *mBio*, Lafont and coauthors characterize the
14 transcriptome of oysters treated with poly I:C. This immune stimulator induced genes related
15 to the interferon and apoptosis pathways. This response overlaps with the response to viral
16 infection and high expression levels of potential effector genes is maintained up to four
17 months. This work opens the door to the characterization of the phenomena of immune
18 priming in a poorly studied invertebrate model. It also highlights the importance of IFN-like
19 responses for invertebrate antiviral immunity.

20 **COMMENTARY**

21 Viral infectious diseases cause significant economic losses to aquacultures. While bacterial
22 infections are commonly controlled by the use of antibiotics, we still lack effective antivirals.
23 Viruses are obligate intracellular parasites, offering few targets for specific inhibitors. As a
24 consequence, prophylaxis is the most effective way to control viral infections. In mammals
25 and other vertebrates, prophylactic strategies against viruses are based on specific priming of
26 the adaptive immune systems by vaccination. However, invertebrates do not have the
27 adaptive arm of the immune system, relying solely on innate immunity. Development of
28 prophylactic measures in economically important invertebrate species requires a better
29 understanding of their antiviral defenses.

30

31 Innate immunity is the first line of defense against viruses in all animals. In vertebrates,
32 production of interferon (IFN) family cytokines is the hallmark of the innate antiviral response.
33 These are produced by virus-infected cells upon the recognition of different pathogen
34 associated molecular patterns (PAMPs), such as double-stranded RNA (dsRNA), a hallmark of
35 RNA virus replication or convergent transcription in DNA viruses. dsRNAs are recognized by
36 specialized receptors such as TLR3, MDA5 or RIG-I, depending on PAMP size, concentration
37 and intracellular localization. Recognition of dsRNA triggers intracellular cascades that
38 culminate in the translocation to the nucleus of interferon regulatory factors (IRF) among
39 other transcription factors. This results in the production of interferons and induction of a
40 large array of antiviral genes to control the infection.

41

42 In animals devoid of interferons, such as invertebrates, RNA interference (RNAi) is often
43 described as the major antiviral defense (1). Since antiviral RNAi is shared with distantly

44 related eukaryotes (e.g. plants), it is postulated that the interferon response replaced the
45 antiviral function of RNAi in vertebrates (2). However, most experimental work supporting this
46 assumption comes from two main models, the insect *Drosophila melanogaster* and the
47 nematode *Caenorhabditis elegans*, two ecdysozoan protostomes. Ecdysozoa, together with
48 the Lophotrochozoa (mollusks and annelids) and the Platyzoa (e.g. flatworms) form the major
49 subdivisions of the protostomes. Ecdysozoa have repeatedly lost IRFs (3), hence they are not
50 fully representative of the diversity in immune pathways potentially present in protostomes.
51 Other protostomes, including mollusks, not only have multiple IRF genes (3), but also
52 homologs of several other components of the of the IFN pathway including sensors (MDA-5,
53 RIG-1, TLRs), signal transducers (MAVS, STING, MyD88, IkkB), transcription factors (NF-kB/Rel)
54 and effectors (OAS, Viperin, Mx) (4).

55

56 The work directed by Caroline Montagnani sheds new light on the importance of IFN-like
57 responses for invertebrate antiviral immunity. The authors and others had previously shown
58 that treating oysters with dsRNA or polyinosinic:polycytidylic acid (poly I:C) confers a long-
59 term protection against Ostreid Herpesvirus 1 (OsHV-1) (5). This virus causes Pacific oyster
60 mortality syndrome, which represents an important economic burden for shellfish farmers.
61 Here, using RNA sequencing, Lafont *et al.* explore the genome-wide transcriptomic changes
62 triggered by poly I:C, a synthetic dsRNA analog that is a potent agonist of TLR-3 and MDA5 in
63 mammals, and OsHV-1. Poly I:C treatment induced a strong transcriptional response (more
64 than 4000 genes differentially expressed, representing approximately 16% of the annotated
65 transcriptome), with a majority of genes upregulated at one or several timepoints post-
66 treatments. Most interestingly, the majority of genes induced by poly(I:C) are also induced by
67 OsHV-1 infection, suggesting that poly I:C primes an immune response similar to that of the

68 virus, which results in a protective antiviral state. Further supporting this interpretation,
69 Lafont *et al.* observed only minor transcriptional changes following OsHV-1 infection in poly
70 I:C primed oysters. Strikingly, gene ontology analysis revealed a strong enrichment of immune
71 functions among poly I:C induced genes. Many of these genes belong to known antiviral
72 pathways characterized in mammals, such as the IFN (e.g. MDA-5, STING, OAS, Viperin) or
73 apoptosis pathways. If a role of IRF transcription factors in the regulation of these genes can
74 be confirmed, this would suggest that the IFN pathway in vertebrates was grafted to an
75 ancestral antiviral response regulating a repertoire of evolutionarily ancient restriction
76 factors.

77

78 Remarkably, the authors show that induction of several potential antiviral effectors is
79 sustained for several days or even months after stimulation with poly I:C. This sustained
80 induction of an antiviral response is the possible underlying cause of the observed immune
81 priming. Indeed, innate immune priming and memory have been described in several species
82 including invertebrates that lack an adaptive immune system. However, the molecular
83 mechanisms are poorly characterized and is probably of heterogeneous nature (6). The
84 findings reported here provide an interesting model to start to functionally explore the
85 molecular mechanisms at play in immune priming of a long lasting response.

86

87 In light of the potential use of priming with poly(I:C) as a prophylactic strategy in oysters
88 (referred to as “pseudo-vaccination” by the authors), a final question pertains to the potential
89 impact of a persistent expression of antiviral genes on oyster physiology, which may impact
90 productivity. Indeed, even though the authors did not observe an impact of poly I:C treatment

91 alone in the survival of oysters (5), sustained expression of ISGs in humans is associated with
92 a number of diseases known as interferonopathies (10).

93

94 Several questions remain open in this fascinating model. How is the viral genetic material and
95 poly I:C recognized? The oyster RIG-I homolog is able to bind poly I:C in vitro (7), so this is a
96 main candidate for a receptor of dsRNAs. Nevertheless, like in vertebrates, there could be
97 specialized receptors for different PAMPs, and the roles of the multiple TLRs and of the
98 cGAS/STING orthologs remain to be characterized. Would agonists of these pathways yield
99 the same protective effect? The effector mechanisms remain also to be identified. A first
100 interesting point to address would be to determine how broad the protection conferred by
101 poly I:C is, by infecting oysters with other families of viruses. This would imply an effort to
102 explore molluskan viruses, a poorly developed field that deserves further attention. In
103 humans, the functional characterization of hundreds of interferon-stimulated genes already
104 revealed several novel antiviral effectors (8). Others demonstrated that while the core
105 interferome of vertebrates contains the best characterized antiviral proteins, each clade has
106 several specific ISGs whose antiviral function is unknown (9). Given the diversity of
107 invertebrate species, their antiviral defenses constitute an unexploited reservoir of novel
108 antiviral genes with potential relevance to human health.

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116

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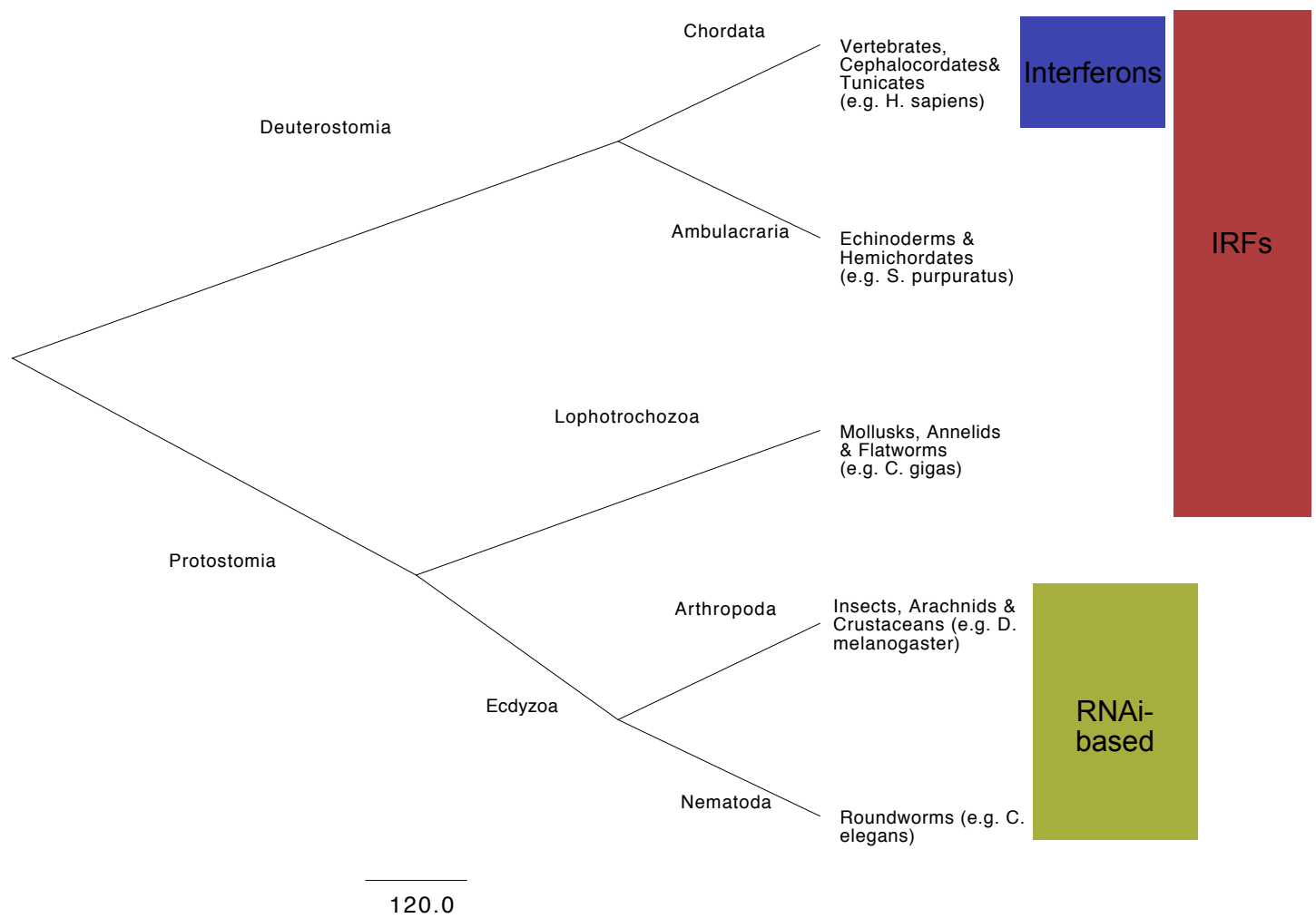


Fig 1 - Simplified metazoan taxonomy of the major animal clades discussed in the text. Invertebrates are not monophyletic, comprising both deuterostomes (e.g. sea-urchins) and protostomes. Innate antiviral defenses are defined mainly for mammalian vertebrates (Chordata), insects (Arthropoda) and roundworms (Nematoda). Production of interferons (IFN), controlled by interferon regulatory factors (IRFs) among other transcription factors, is the hallmark of the vertebrate innate antiviral immunity. Constitutive RNA interference (RNAi) is described as the major antiviral defense in invertebrates, but inducible responses are poorly characterized in these animals. Multiple invertebrate clades (Ambulacraria, Lophotrochozoa and some Arthropods) have homologs for IRFs, which may play a role in antiviral response. The work by Lafont et al. identified an IFN like response in the mollusk *Crassostrea gigas*.