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# Evolution of polydom-like molecules: Identification and characterization of cnidarian polydom (*Cnpolydom*) in the basal metazoan *Hydractinia* ☆

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**Summary**
End sequencing of random BAC clones from a *Hydractinia symbiolongicarpus* (Cnidaria: Hydrozoa) genomic library revealed a gene across a ~37.5 kb region of the *H. symbiolongicarpus* genome sharing highest sequence identity and domain architecture to mammalian polydom that we in turn named cnidarian polydom (*CnPolydom*). Sharing all eight domain types characteristic of polydom and organized in a similar 5′–3′ manner, *CnPolydom* was predicted to contain three additional domain types: PAN, FA58C, and CUB that are characteristic of *CnPolydom*. Expression analysis of *CnPolydom* from *H. symbiolongicarpus* (*Hysy-CnPolydom*) showed upregulation in response to bacterial and primarily fungal challenges, with transcripts produced specifically by a subset of interstitial stem cells (i-cells) and/or neural cells throughout the ectodermal tissue layer of feeding polyps (gastrozooids). This is the first description of a polydom-like molecule outside of Mammalia and provides evolutionary perspective on the ancestral structure and role of this pentraxin family clade.
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## Introduction

It has become increasingly clear that members of the ancestral animal phylum Cnidaria have evolved surprisingly complex genomes and provide novel insights into the extent of genetic conservation and divergence across the metazoan lineages [1–3]. Thus, we can gain comparative insight into the evolution of animal immune systems, and the individual building blocks from which they are comprised, by assessing

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the repertoire of immune genes in these ancestral animals having a true tissue level of organization (Eumetazoa). Within the Cnidaria, class Anthozoa and Hydrozoa are the most ancestral of the four currently recognized classes [4,5]. Genes encoding immune-related molecules from members of the Hydrozoa and Anthozoa are recent targets of research to assemble the complexity of basal eumetazoan immune components [6], and the hydrozoan *Hydractinia* has proven an effective study organism in this pursuit [7–9]. These colonial cnidarians typically grow as an encrustation on gastropod shells occupied by hermit crabs and are comprised of many polyps, including gastrozooids (feeding polyps) and gonozooids (reproductive polyps), that are interconnected via a gastrovascular system of stolons embedded in a stolonal mat [10,11]. *Hydractinia* are diploblastic, having an ectodermal and endodermal cell layer separated by an acellular mesoglea. Being the most basal eumetazoans, hydrozoans have true tissue level organization and three stem cell lineages from which all of their differentiated cell types arise: ectodermal, endodermal, and interstitial stem cells [12,13]. *Hydractinia* have become a model, colonial hydrozoan representative of Cnidaria for developmental, reproductive, and immunological research [11].

Cnidarian immune research is beginning to reveal novel immune-related genes in cnidarians [9], in addition to genes that are well conserved through higher metazoan taxa. Such conserved immune-related components include a complement 3-like gene described from the anthozoan coral *Swiftia exserta* [14], known to be central to the opsonic function of the complement system in vertebrates, and in both Hydrozoa and Anthozoa conserved genes containing membrane attack complex/perforin (MAC/PF) domain have been reported [6]. In addition, many components of the Toll/TLR pathway have been identified in both Anthozoa [15] and Hydrozoa [6,16], including transmembrane receptors encoding toll/interleukin 1 receptor (TIR) domains (Hydrozoa and Anthozoa) and multiple leucine rich repeat (LRR) domains (Anthozoa only), intracellular signaling cascade components including MyD88 genes containing the functional DEATH domain, and transcription factors of the Rel/NF-$\kappa$B and NFAT gene family that initiate effector genes such as antimicrobial peptides. Lacking in many of these studies, however, is assessment of the immunologic relevance of these genes within the Cnidaria.

Pentraxin molecules are a well characterized family of immune components known for their highly conserved structural motif, the pentraxin domain, and for their function as recognition and effector molecules in the innate immune responses of both vertebrates and invertebrates (reviewed in [17,18]). One group of pentraxin molecules includes C-reactive protein (CRP) and serum amyloid P component (SAP), the prototypical short pentraxin proteins of the acute-phase response classically described in mammals and known to bind a variety of microorganisms including fungi, yeast, and bacteria [19,20], as well as common membrane moieties including phosphorylcholine, phosphorylethanolamine, and lipopolysaccharide (LPS) [21–24]. These proteins polymerize and have direct opsonic and complement activation ability [25,26]. A diverse repertoire of CRP-like and SAP-like short pentraxin molecules having similar antimicrobial roles in the hemolymph of three horseshoe crab taxa have also been well studied [27–29]. A second group of pentraxins, known as the long pentraxins, was first identified with the cloning of human pentraxin 3 (PTX3) [30]. PTX3 is also an acute phase protein and has been linked most importantly to its role in mediating fungal recognition and phagocytosis [31,32].

A new member of the pentraxin family has been recently described from mammals, but its function and the extent of its evolutionary conservation in other taxa is unknown. Polydom was first identified and described by Gilgès et al. in 2000 from mouse [33]. *Polydom* was originally amplified from total RNA of a murine haematopoiesis-sustaining bone marrow stromal cell line (MS-5) using degenerate EGF domain-specific primers [33]. The full-length mouse mRNA encodes a 3567aa protein predicted to include a novel combination of domains including an N-terminal von Willebrand factor A (VWA) domain, 2 hyalin repeat domains (HYR), 10 epidermal growth factor repeats (EGF), 34 complement control protein (CCP) domains, and a single pentraxin domain (PTX) at its core, making polydom a member of the pentraxin family of lectins. Based on this domain composition, *polydom* has recently been given the additional name *SVEP*1 for sushi, von Willebrand factor A, epidermal growth factor, pentraxin molecule 1 [34].

Though largely unknown, data on polydom are helping to elucidate potential functional roles of the polydom protein in humans and mice. *Polydom* expression has been shown in several tissue types including skeletal tissues (bone and periosteum) of human and mouse, with protein expression demonstrated on the surface of human stromal cells (osteoblasts) where they act as cell adhesion molecules (CAMs) [34]. Several studies have shown one important location of *polydom* expression is in the placenta of human and mouse. In human, the transcript level of *polydom* from basal plate tissue of the placenta, where allogeneic interaction occurs between maternal and fetal cells, more than doubles once a pregnancy comes to term (37–40 weeks) compared to polydom levels during pregnancy (14–24 weeks) [35]. Although placental expression of *polydom* has been shown to be substantial in these mammals, polydom-like sequences are also predicted from non-placental vertebrate taxa including chicken and zebrafish, as well as invertebrate taxa, suggesting the placental role for polydom is a derived function in mammals.

Using a *Hydractinia symbiolongicarpus* BAC library to randomly examine their genome for unknown genes of potential immunologic relevance, we identified a modular gene comprised of many domain types known for their association with immune molecules including: VWA, EGF, CCP, PTX, PAN, FA58C, and CUB domains. Using primers designed from the genomic sequence we performed RT-PCR and amplified partial cDNAs to confirm transcription of this gene from *H. symbiolongicarpus* and its sister species *Hydractinia echinata*, and have determined the encoded gene's exon-intron structure within the *H. symbiolongicarpus* genome according to the corresponding cloned cDNA and predicted ORFs. The expressed gene, comprised of 12 distinct domain types, shared highest identity to the polydom gene from human ($E = 1e{-}99$; EMBL CAH74139) and we have in turn named it cnidarian polydom (*CnPolydom*), and identify the gene from *H. symbiolongicarpus* and *H. echinata* as *Hysy-CnPolydom* and *Hyec-CnPolydom*,