ISJ 6: 106-113, 2009 ISSN 1824-307X

REVIEW

The innate immunity in the cnidarian *Hydra vulgaris*

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Accepted July 29, 2009

Abstract

Hydra vulgaris is currently receiving increased attention as a genetically tractable invertebrate model system for studying important processes of life such as the innate immune defense. Similar to complex animals, *H. vulgaris* polyps respond to injury by abrupt muscle contraction, by limited escape behavior, and by healing the damaged tissue. Simultaneously, cellular processes such as phagocytosis and programmed cell death as well as the massive production of antimicrobial peptides are induced. Recent studies identified several molecular pathways controlling these responses; however, the interdependence of innate immunity and, for example, regeneration and tissue remodeling is not well elucidated yet. *H. vulgaris* belongs to the Cnidaria representing the phylogenic sister group of bilaterian animals; hence, a better understanding of evolutionarily conserved as well as *Hydra*/Cnidaria-specific immune responses will provide deep insight into both origin and evolution of the animal innate immune system.

Key Words: Cnidaria; danger signaling; *Hydra vulgaris*, innate immunity; regeneration; model organism

Introduction

A major evolutionary split occurred in the animal kingdom between so-called basal animals, which include Cnidaria, and the Deuterostomia/Protostomia, which include most other animals (Fig. 1) (Finnerty *et al*., 2004). The freshwater cnidarian *Hydra vulgaris* has a simple body plan with two cell layers, the ectoderm and the endoderm, separated by an extracellular matrix (mesoglea). *Hydra* is prominent for its ability to regenerate a complete organism from small body fragments. Therefore, *H. vulgaris* has widely been used as feasible model system to study pattern formation, cell differentiation, morphogenesis, and regeneration (Trembley, 1744; Campbell, 1967; Gierer *et al*., 1972; Bode *et al*., 1986; Holstein *et al*., 1991; Bosch, 1998; Hassel, 1998; Galliot and Schmid, 2002; Steele, 2002; Bode, 2003; Holstein *et al*., 2003; Bosch, 2007). This review focuses on recent studies investigating the innate immune system in *H. vulgaris*.

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Hydra vulgaris as model system to study innate immunity

In higher animals, immunity is a complex and highly developed system of specialized cells and organs that protects an organism against bacterial, parasitic, fungal, and viral infections. Besides the conserved innate immunity, the adaptive or acquired immunity is present in vertebrates representing a novel evolutionary acquirement which enables the somatic generation of highly variable T-cell receptors and B-cell derived antibodies by recombination-activating gene dependent genomic rearrangements (Germain, 2004; Akira *et al*., 2006; Medzhitov, 2007). These molecules provide pathogen-specific antigen recognition and create the so-called immunological memory. Invertebrate immunity shows many parallels to the innate immunity of vertebrates, which complements the adaptive immunity. It includes cellular phagocytosis, antiviral RNA-interference, and killing of pathogens by antimicrobial peptides, lysozymes, and reactive oxygen species (Boman, 2003; Kim and Ausubel, 2005; Cherry and Silverman, 2006; Lemaitre and Hoffmann, 2007) (Fig. 1).

Both vertebrate and invertebrate innate immunity recognize invading pathogens by hereditary pattern recognition receptors including

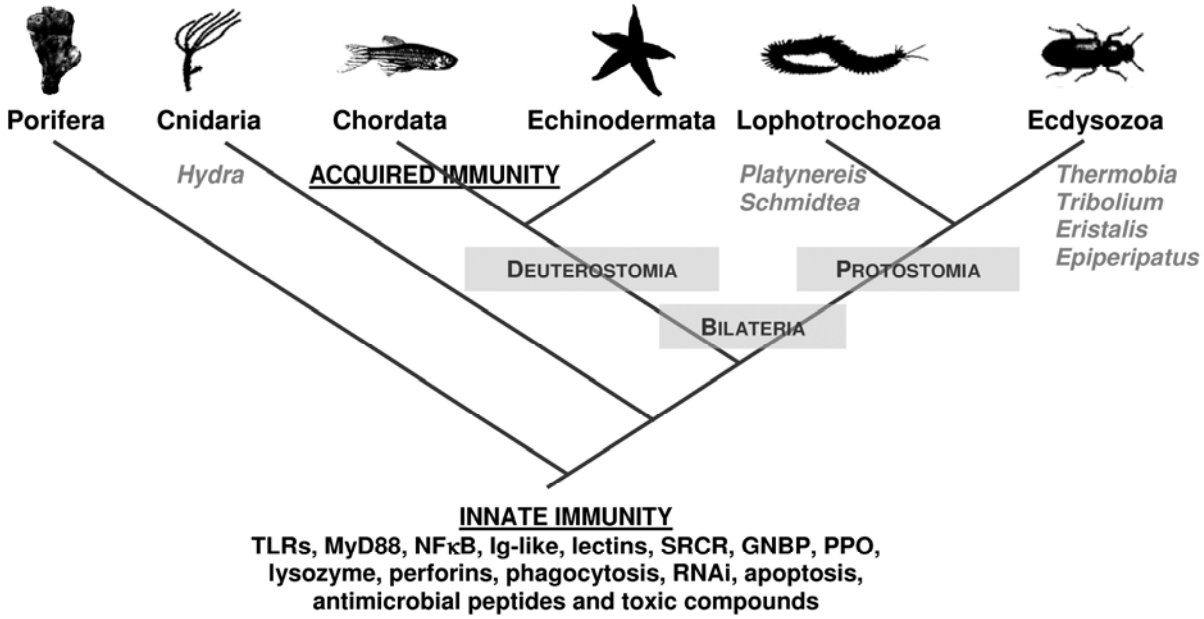

Fig. 1 Origin and evolution of immunity in Metazoa. Many mechanisms of innate immunity such as Toll-like receptors (TLR), RNA interference (RNAi), antimicrobial peptides and toxic compounds are conserved across all animals. However, some aspects such as acquired immunity have only been evolved in vertebrates. Several animal species that we examined for immune inducible genes to further understanding of the evolution of innate immunity are written in gray (Altincicek and Vilcinskas, 2007a, b, c, 2008b, c; Altincicek *et al.*, 2008). NFκB, nuclear factor kappa B, MyD88, myeloid differentiation primary response gene 88; Ig-like, immunoglobulin-like; SRCR, scavenger receptor cysteine rich; GNBP, Gram-negative bacteria-binding protein; PPO, prophenoloxidase.

Toll receptors, peptidoglycan recognition proteins, and lectins which bind to microbial components such as bacterial lipopolysaccharides, peptidoglycans, or fungal ß-1,3 glucans (Royet, 2004; Akira *et al*., 2006; Medzhitov, 2007). Moreover, immune related signaling is achieved in animals ranging from sponges to humans mainly by myeloid differentiation primary response gene 88 (MyD88), nuclear factor kappa B (NFκB)-like factors, and stress-activated protein kinases such as p38 and JNK (Böhm *et al*., 2002; Wiens *et al*., 2005; Akira *et al*., 2006; Wiens *et al*., 2007) (Fig. 1). This suggests a shared root for invertebrate and vertebrate immune pathways conserved over hundreds of millions of years.

To elucidate origin and evolution of animal innate immunity, immune defense reactions have been studied in basal animals such as poriferans and cnidarians. Recently, methods like dsRNA-mediated gene silencing (Lohmann *et al*., 1999; Chera *et al*., 2006; Miljkovic-Licina *et al*., 2007) and of transgenesis allowing stable genetic manipulation have been established in *H. vulgaris* (Wittlieb *et al*. 2006; Khalturin *et al*., 2007). Hence, *H. vulgaris* represents a complementary invertebrate model system to the fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans* for studying important processes of life including the innate immune defense. Moreover, whole genome sequences have become available for two cnidarians, the hydrozoan *Hydra magnipapillata* and the anthozoan *Nematostella vectensis* (Technau *et al.*, 2005). This allowed recent comparative investigations of gene sets regarding innate immunity proteins in these two basal eumetazoans (Hemmrich *et al.*, 2007; Miller *et al*., 2007).

Regarding the use of *H. vulgaris* as model system to study innate immunity, it should be mentioned that numerous *Hydra* species live in mutualistic symbiosis with algae (Pardy and Heacox, 1976). Moreover, symbiosis with algae seems to represent a common feature of the *Hydra* group since symbiosis can be experimentally induced in non-symbiotic *Hydra* species (Rahat and Reich, 1984, 1985, 1986). A tight association also exists with bacterial symbionts or commensals (Wilkerson, 1980; Rahat and Dimentman, 1982; Fraune and Bosch, 2007; Fraune *et al*., 2009) indicating that the immune system of *Hydra* polyps is capable to distinguish between beneficial and pathogenic microbes. Theory predicts that *Hydra* may induce sophistically regulated and probably specific immune responses, which control interacting microbes. Yet, specific receptors or signaling pathways have not been identified, but it is obvious that the evolutionarily conserved programmed cell death (Cikala *et al*., 1999; Böttger and Alexandrova, 2007) plays a major role in controlling mutualistic symbionts or pathogens probably similar to observations from other cnidarian species (Dunn *et al*., 2002; Dunn *et al*., 2004; Ainsworth *et al*., 2007; Dunn, 2009; Dunn and Weis, 2009).

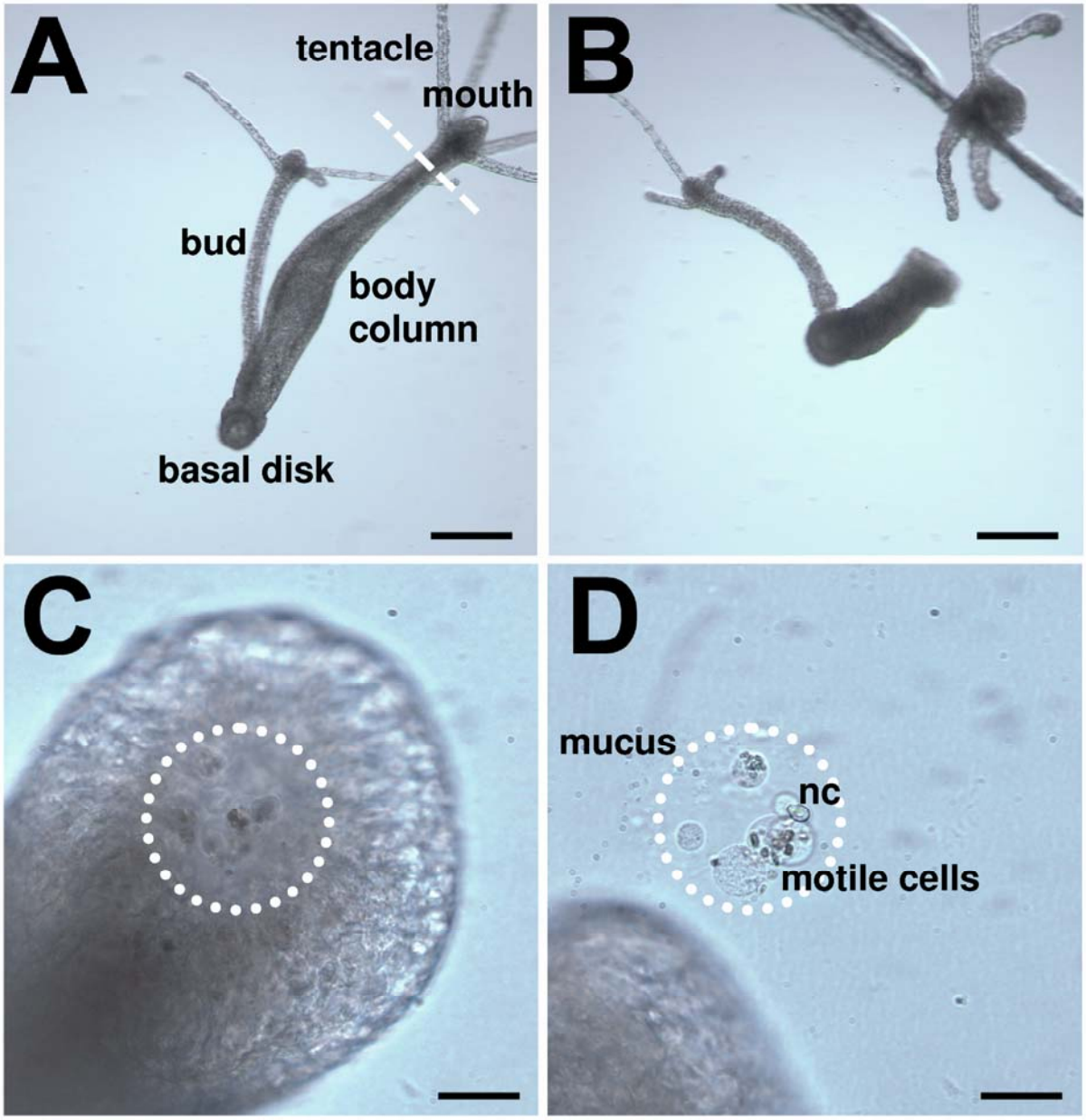

Fig. 2 Early phase responses of *H. vulgaris* polyp to bisection: (A) *H. vulgaris* is organized as body column with a mouth and ring of tentacles at the apical pole and a foot process with a basal disk at the distal pole. Buds are formed for asexual reproduction. (B) Bisection of a polyp results in abrupt muscle contraction and subsequently in limited escape behavior. (C) The contraction reduces the area of injury to a minimum which is indicated by a circle. (D) Within several minutes post bisection mucus is produced and motile cells appear at the wounding site which phagocytize debris form damaged cells. nc, nematocyst. Bars = 200 µm (A, B); 20 µm (C, D).

Immunorecognition and effector molecules

H. vulgaris polyps show many parallels to complex animals regarding to their innate immune reactions. Bisection of *H. vulgaris* polyps, for example, leads to an abrupt muscle contraction and escape reaction (Figs 2A, B). These behavioural reactions are accompanied by the massive production of mucus at the wounding site and the appearance of phagocytic cells eliminating cell debris and dying cells (Figs 2C, D). Significantly, homogenates obtained from bisected animals exhibited a significant higher level of antimicrobial activity than homogenates obtained from untreated animals as determined by an inhibition zone assay with live bacteria (Fig. 3). This is in line with recent observations that the *Hydra* innate immune system senses tissue injury itself or along with bacterial contamination from the environment to induce the massive production of antimicrobial peptides along

with further potential bactericidal or bacteriostatic molecules (Altincicek and Vilcinskas, 2008a; Jung *et al*., 2008; Bosch *et al*., 2009).

The finding that the *Drosophila* transmembrane protein Toll, which is essential for the development of embryonic dorsoventral polarity in the fruit fly, also mediates immune responses to infection had a pioneering role in the identification of Toll-like receptors as evolutionarily conserved and essential regulators of immunity (Beutler, 2004). Mammalian Toll-like receptors recognize a highly divergent collection of ligands such as lipoproteins, bacterial lipopolysaccharide and peptidoglycans, fungal zymosan, plant diterpene paclitaxel, viral proteins, and endogenous molecules such as heat-shock proteins and nucleic acids, all of which have different structures (Akira *et al*., 2006; Karin *et al*., 2006). In *Nematostella*, Toll-like receptors and a NFκB homolog have been identified but, surprisingly, not in *Hydra* suggesting that a substantial immune gene loss or significant gene diversification of these factors have occurred during *Hydra* evolution (Miller *et al*., 2007). Consistent with this hypothesis, a recent study indicated that in *Hydra* instead of typical Toll-like receptors two related Toll-IL-1 receptor (TIR) domain transmembrane proteins interact with leucine-rich repeat and epidermal-growth factor domain transmembrane proteins to induce the expression of antimicrobial peptides (Bosch *et al*., 2009); however, a corresponding transcriptional activator of this signaling pathway has yet not been identified.

Our recent studies with *H. vulgaris* revealed several immune-relevant inducible genes including, for example, a potential antimicrobial peptide, major vault protein and p47phox protein or neutrophil cytosolic factor 1 (Altincicek and Vilcinskas, 2008a). In mammals, major vault protein has been demonstrated to be essential for optimal epithelial cell phagocytosis of the pathogenic bacterium *Pseudomonas aeruginosa* (Kowalski *et al*., 2007). Phagocyte NAPDH-oxidase (Phox) is responsible for the generation of superoxide with secondary production of other microbicidal reactive oxygen species (ROS) in human neutrophils and macrophages. Phox consists of the catalytic subunit gp91phox, along with the regulatory subunits p22phox, p67phox, p40phox, the small GTPase Rac, and p47phox or neutrophil cytosolic factor 1 (El-Benna *et al*., 2009). Since both molecules, major vault protein and p47phox protein, are reported to be important in mammalian immunity to eliminate invading pathogens investigation of their counterparts in *Hydra* immune responses may further our understanding of human immune defense reactions.

Injury induces immune responses and regeneration processes

The ability to respond to injury and to repair tissue is a fundamental property of all multicellular organisms. Injury of the vertebrate skin, for example, initiates a complex sequence of events involving inflammation as well as the formation and remodeling of new tissue (Schäfer and Werner,

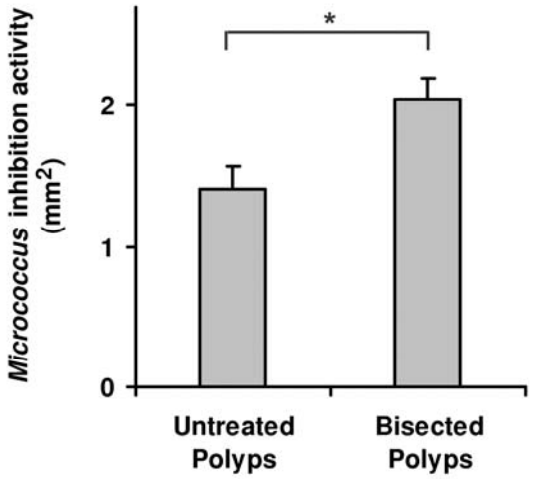

Fig. 3 Bisection induces expression of antimicrobial factors in *H. vulgaris* polyps. Homogenates obtained from 10 animals 24 h post bisection exhibited a significant higher level of antimicrobial activity than homogenates obtained from 10 untreated animals. Antibacterial activity was determined with live *Micrococcus luteus* as described (Altincicek and Vilcinskas, 2008c). Results represent mean values of three independent repetitions ±SD. Statistical significance was determined by Student's t-test ($p<0.01$).

2007). At the wounding site, multiple biological pathways immediately become activated and are synchronized to respond. The mammalian immune system is triggered by both microbial pattern molecules and endogenously derived danger signals such as extracellular heat shock proteins, uric acid, and HMGB1, which are released during tissue injury (Matzinger, 2002; Akira *et al*., 2006; Karin *et al*., 2006). Similarly, *Hydra* is capable in sensing microbial molecular pattern such as lipopolysaccharide (Altincicek and Vilcinskas, 2008a; Bosch *et al*., 2009) and flagellin (Bosch *et al*., 2009) as well as danger molecules derived from dying or damaged cells such as extracellular nucleic acids and uric acid to induce expression of antimicrobial peptides (Bosch *et al*., 2009).

In vertebrates, wound healing requires Dickkopf and Wnt/β-Catenin signaling and, additionally, the action of TGF-β/Bmp and matrix metalloproteinases (MMPs) (Stoick-Cooper *et al*., 2007). Homologs of all of these factors have also been described to regulate *Hydra* regeneration processes (Galliot and Schmid, 2002; Holstein *et al*., 2003). Recent studies, for example, demonstrated that the Wnt and TGF-β/Bmp signaling pathway components are transcriptionally up-regulated early during regeneration in *Hydra* (Holstein *et al*., 2003) and that the *Hydra* Dickkopf-like protein expression that antagonizes Wnt/β-Catenin signaling is stimulated by the injury signal itself (Holstein *et al*., 2003). It has furthermore been shown that *Hydra* astacin-class metalloproteinases are crucial for both foot and head regeneration (Yan *et al*., 2000a, b) and that

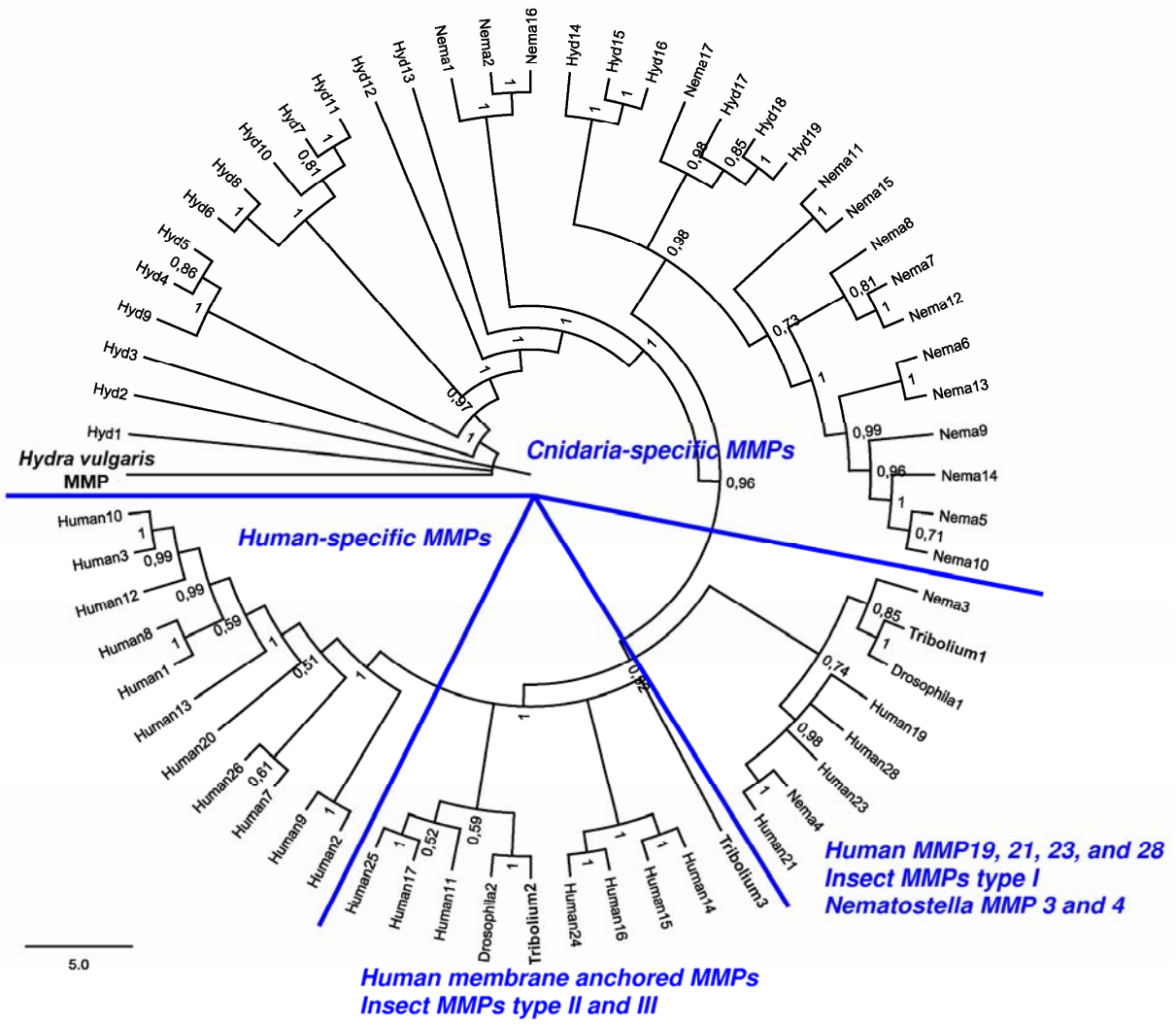

Fig. 4 Phylogenic analysis of Cnidaria MMPs. Phylogenic reconstruction was performed with the software package MrBayes 3.1.2 similar as described (Knorr *et al.*, 2009). The model with the overall highest posterior probability was WAG. The average standard deviation of split frequencies at 10^6 generations was 0.01 and therefore indicated that the two chains that were run converged on similar results. The tree was drawn with FigTree (tree.bio.ed.ac.uk/software/figtree). Posterior probabilities are plotted at the nodes, which can be interpreted as the probability that the tree or clade is correct. The scale bar represents the substitutions per site. Accession numbers of investigated MMPs are as follows: *T. castaneum* (Tribolium1, XP_968822; Tribolium2, XP_969495; Tribolium3, XP_972146); *D. melanogaster* (Drosophila1, NP_523852; Drosophila2, NP_995788), *Homo sapiens* (Human19, Q99542; Human28, Q9H239; Human11, P24347; Human21, Q8N119; Human23, O75900; Human17, Q9ULZ9; Human25, Q9NPA2; Human14, P50281; Human15, P51511; Human16, P51512; Human24, Q9Y5R2; Human20, O60882; Human12, P39900; Human13, P45452; Human1, P03956; Human8, P22894; Human3, P08254; Human10, P09238; Human7, P09237; Human26, Q9NRE1; Human2, P08253; Human9, P14780); *H. vulgaris* MMP (AAD45804); *H. magnipapillata* (Hyd1, XP_002158607; Hyd2, XP_002163047; Hyd3, XP_002163794; Hyd4, XP_002160477; Hyd5, XP_002170324; Hyd6, XP_002164979; Hyd7, XP_002168433; Hyd8, XP_002168462; Hyd9, XP_002155089; Hyd10, XP_002161129; Hyd11, XP_002166835; Hyd12, XP_002168334; Hyd13, XP_002155205; Hyd14, XP_002154985; Hyd15, XP_002165116; Hyd16, XP_002163948; Hyd17, XP_002164594; Hyd18, XP_002165203; Hyd19, XP_002163814); *N. vectensis* (Nema1, XP_001640669; Nema2, XP_001640565; Nema3, XP_001633230; Nema4, XP_001629775; Nema5, XP_001636910; Nema6, XP_001635715; Nema7, XP_001640426; Nema8, XP_001638726; Nema9, XP_001640819; Nema10, XP_001630740; Nema11, XP_001626901; Nema12, XP_001640427; Nema13, XP_001622031; Nema14, XP_001630704; Nema15, XP_001642037; Nema16, XP_001635184; Nema17, XP_001628710).

a *Hydra* matrix metalloproteinase (MMP) represents a central effector molecule in extracellular matrix degradation, epithelial morphogenesis, and probably in cell differentiation (Leontovich *et al*., 2000; Sarras *et al*., 2002; Shimizu *et al*., 2002).

In humans, more than 20 different MMPs have been identified which degrade virtually all extracellular matrix proteins and release or degrade bioactive fragments and growth factors (Page-McCaw *et al*., 2007). They influence fundamental biological and pathological processes like embryonic development, angiogenesis, tissue homeostasis and morphogenesis, wound repair, inflammation, and tumor progression (Page-McCaw *et al*., 2007). The recently investigated *H. vulgaris* MMP (Leontovich *et al*., 2000; Sarras *et al*., 2002; Shimizu *et al*., 2002) has a well-conserved overall domain structure to that of their invertebrate and vertebrate counterparts. *H. vulgaris* MMP includes a pro-peptide sequence containing a conserved inhibitory cysteine switch sequence and the catalytic domain with the conserved HEXXHXXGXXH sequence critical for binding the catalytically active zinc ion (Nagase and Woessner, 1999). Interestingly, examination of the cnidarian whole genome sequences revealed that 19 *H. magnipapillata* and 17 *N. vectensis* MMP homologs exist which most of them clade together with the identified *H. vulgaris* MMP (Fig. 4). These observations suggest that MMPs fulfill complex and overlapping functions in Cnidaria probably similar to mammalian MMPs (Page-McCaw *et al*., 2007). Interestingly, two *Nematostella* MMPs grouped together with human MMP19, 21, 23, 28 and insect MMP1s indicating possible evolutionarily conservation of these MMPs in *N. vectensis* and loss in *H. magnipapillata* (Fig. 4). MMPs have further been recognized as important modulators of immunity in both mammals (Parks *et al*., 2004; Vanlaere and Libert, 2009) and insects (Altincicek and Vilcinskas, 2008c; Knorr *et al*., 2009); hence, we believe that their investigation in *Hydra* will help to unravel the complex interdependence of the immunity with regeneration processes.

Future priorities in the field of studying *Hydra* innate immunity

Goals in the field of studying innate immunity in the *H. vulgaris* model system will be the elucidation of both genetic mechanisms driving host-parasite as well as host-symbiont co-evolution and principles of epithelial immune mechanisms in an animal that lacks a coelomic cavity. Examination of the *Hydra* immunity may further help to understand interactions of reef-building Cnidaria with their pathogens as well as their symbionts (Bosch, 2008) helping to keep these ecosystems alive. In this context, however, it should also be noted that the depth of the split between the cnidarian classes Anthozoa (*Nematostella*) and Hydrozoa (*Hydra*) has been determined to be as great as that between Protostomia and Deuterostomia (Miller and Ball, 2008). This observation indicates that immune mechanisms may be highly derived within Cnidaria as, for example, found for the Toll and NFκB signaling as mentioned above.

In conclusion, the investigation of innate immune defense of basal animals with *H. vulgaris* as a genetically tractable model system is an exciting field, which will give valuable insight into the evolution of animals. Combining mechanistic understanding of immunity along with processes of regeneration and of development will be, without doubt, a fruitful union.

Acknowledgements

I want to thank Monika Hassel (Philipps University of Marburg, Germany) for kindly providing me with *Hydra vulgaris* (Zürich strain) and for helpful comments. I am grateful to Andreas Vilcinskas (Justus-Liebig-University of Giessen, Germany) and Katja Altincicek for critical reading of the manuscript. Financial support by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) with the Heisenberg fellowship (AL902/4-1) is greatly appreciated.

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