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Peptide signaling in *Hydra*

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ABSTRACT Peptides play a number of crucial roles as signaling molecules in metazoans. In order to elaborate a more complete picture of the roles played by peptides in a single organism, we launched the "*Hydra* Peptide Project". For this project, we used *Hydra magnipapillata*, a species belonging to Cnidaria, one of the most basal metazoan phyla, and using a peptidomic approach, we systematically identified a number of peptide signaling molecules, their encoding genes and their functions. In this article, we report the peptides isolated from *Hydra* and other cnidarians, as well as their synthesis, processing and release from the cells to the target. Possible peptide signaling pathways are overviewed and finally we discuss the evolution of the peptide signaling system.

KEY WORDS: *signaling peptides, Hydra, GPCR, signaling pathway, evolution*

Introduction to the *Hydra* peptide project

Peptides play a number of crucial roles as signaling molecules in the cell activities of metazoans. For example, neuropeptides regulate a variety of physiological functions as neurotransmitters or hormones. Peptides of non-neuronal origin also mediate cell-to-cell communication as hormones and maintain homeostasis of the organism. Cnidaria, one of the most basal metazoan phyla possesses the most primitive nervous system. It is believed that cnidarian ancestors acquired the first nervous system. In this context it is important to investigate neurotransmission in cnidarians. Grimmelikhuizjen and his coworkers (1992) have isolated a group of peptides related to molluscan FMRFamide (RFamide) from a variety of cnidarians and advocated that cnidarian neurotransmission is mediated exclusively by peptides. On the other hand, we launched a project in which peptide signaling molecules were systematically identified in *Hydra*, a cnidarian model organism with established infrastructures (Takahashi *et al*., 1997; see Fujisawa, 2008 for review). In this study peptides with less than 5 kDa in molecular weight were targeted. We identified a variety of peptides that are categorized into two groups. One group consists of neuropeptides and the other epitheliopeptides. In addition to neurotransmission, some neuropeptides trigger metamorphosis of planula larvae of a marine hydrozoan, *Hydractinia echinata* (Leitz *et al*., 1994; Leitz and Lay, 1994) and anthozoans, reef building corals (Iwao *et al*., 2004). As described later, a *Hydra* neuropeptide regulates neuron differentiation. Epitheliopeptides that are derived from epithelial cells most notably contribute to pattern formation and morphogenesis (Fujisawa, 2003). Antimicrobial peptides in *Hydra* are also produced from epithelial cells (Augustin and Bosch, 2010). In this article antimicrobial peptides and peptide toxins are not dealt with because they are somewhat out of scope of this review on signaling peptides.

In cell-to-cell communication, a peptide signal is commonly relayed via cell surface receptor. Receptors for peptide ligands are in most cases G-protein coupled 7 transmembrane receptors (GPCRs). Although GPCRs in cnidarians are poorly understood, a general scheme of signal transduction will be discussed. Peptide signaling is also discussed in the light of evolution.

Peptides identified in cnidarians

Grimmelikjuizen and his co-workers have isolated a number of neuropeptides from different kinds of cnidarians: from the anthozoans *Anthopleura elegantisma* and *Renilla koellikeri*; from the hydrozoans *Polyorchis penicillatus* and *Hydra magnipapillata*; from the scyphozoan *Cyanea lamarckii* (Table 1). Their neuropeptides include KAamide, RIamides, RNamides, RWamides, RPamides and RFamides.

We had undertaken "*Hydra* peptide project" aiming at identifying all the signaling peptides in *Hydra* (see Fujisawa, 2008 for review). Neuropeptides we identified biochemically are also listed in Table 1. Most of the *Hydra* peptides are neurotransmitters and/or neuromodulators. They may act directly on epithelial muscle

Abbreviations used in this paper: GPCR, G-protein coupled receptor.

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cells or indirectly via other neurons. The ectodermal epithelial cells have muscle processes running longitudinally, while endodermal epithelial cells contain circular muscle processes. Thus, in order to achieve body elongation endodermal muscles should contract and ectodermal muscles relax. To contract the body, ectodermal muscle processes contract while the endodermal ones relax. The availability of epithelial *Hydra* that is essentially made of epithelial cells (see in this issue the review by Shimizu, 2012) makes it easy to identify the target muscles of a neuropeptide. For example, if a peptide induces contraction of epithelial *Hydra*, it acts directly on the ectodermal muscle processes. If a peptide has no effect on epithelial *Hydra* but induces contraction of normal *Hydra*, the peptide very likely acts on other neurons to induce secretion of some peptide that in turn causes ectodermal muscle contraction. Among neuropeptides, Hym-355 is unique in that it has no effect on muscle contraction but is rather positively involved in neuron differentiation (Takahashi *et al*., 2000; see below). In addition to the peptides listed in Table 1, several novel neuropeptides by using HPLC-tandem Mass-spectrometry (LC-MS/MS) have been identified (Takahashi *et al*, unpublished).

Peptides derived from epithelial cells are referred to as epitheliopeptides and they are primarily involved in pattern formation or morphogenesis in *Hydra*. Their list has been published elsewhere (Fujisawa, 2003, 2008). Some of the epitheliopeptides (Hym-323, Pedin/Hym-330 and Pedibin/Hym-346) are involved in foot formation (Hoffmeister, 1996; Grens *et al*., 1999; Harafuji *et al*.,

TABLE 1

BIOCHEMICALLY IDENTIFIED CNIDARIAN NEUROPEPTIDES

Species	Peptide	Structure	Function	Refs
Anthopleura elegantissima	KAamide	L-3-phenyllactyl FKAa	Inhibition of muscle contraction	a), b)
(Anthozoa)	RIamide I	L-3-phenyllactyl YRIa	Inhibition of muscle contraction	b,c)
	II	YRIa		c)
	RNamide I	L-3-phenyllactyl LRNa	Induction/inhibition of muscle contraction	d,e)
	II	LRNa		f)
	RWamide I	<QSLRWa	Muscle contraction	g,h)
	II	<QGLRWa	Muscle contraction	h,i)
	RPamide I	LPPGPLPRPa		j)
	II	<QNFHLRPa		k)
	III			f)
	IV			f)
	V			f)
	RFamide family		Induction/inhibition of muscle contraction	l, m)
Renilla koellikeri **(Anthozoa)**	RFamide	<QGRFa		n)
Cyanea lamarckii	RFamide I	<QWLRGRFa		o)
(Scyhozoa)	II	<QPLWSGRFa		o)
	III	GRFa		o)
Polyorchis penicilltus	RFamide I	<QLLGGRFa		p)
(Hydrozoa)	II	<QWLKGRFa		q)
Hydra magnipapillata	RFamide I	<QWLGGRFa		r)
(Hydrozoa)	II	<QWFNGRFa		r)
	RFamide III/IV	(KP)HLRGRFa	Enhance body pumping	r)
	GLWamide family		Induction of metamorphisis of planula larvae	s,t,u)
Anthopleura elegantissim	Metamorphosisn A	<QQPGLWa		s)
Hydra magnipapillata	Hym-53	NPYPGLWa	Enhance bud detachmen t	t)
	Hym-54	GPMTGLWa	Enhance bud detachmen t	t)
	Hym-248	EPLPIGLWa	Enhance bud detachment; Body elongation	t)
	Hym-249	KPIPGLWa	Enhance bud detachmen t	t)
	Hym-331	GPPPGLWa	Enhance bud detachmen t	t)
	Hym-338	GPP^{h}PGLWa[1)]	Enhance bud detachmen t	t)
	Hym-370	KPNAYKGKLPIGLWa	Enhance bud detachmen t	u)
	Hym-1071	KPPWRGGM(O)W		v)
	Hym-176	APFIFPGPKVa	Contraction of peduncle	w)
	Hym-357	KPAFLFKGYKPa	Contraction of whole body	w)
	Hym-690	KPLYLFKGYKPa	Contraction of whole body	v)
	Hym-355 family			
	Hym-355	FPQSFLPRGa	Enhance neuron differentiation	x)
	FRamide family			
	Hym-65 (FRamide 1)	IPTGTLIFRa	Body elongation	y)
	Hym-153 (FRamide-2)	APGSLLFRa	Body contraction	y)

1) The 4th residue proline is hydroxylated. <Q designates C-terminal pyroglutamate. For references see: a) Nothaker *et al*., 1991a; b) McFarlane *et al*., 1993; c) Nothaker *et al*., 1991b; d) Grimmelikhuijzen *et al*., 1990; e) McFarlane *et al*., 1992; f) Grimmelikhuijzen *et al*., 2002; g) Graf and Grimmelikhuijzen, 1988a; h) McFarlane *et al*., 1991; i) Graf and Grimmelikhuijzen, 1988b; j) Carstensen *et al*., 1992; k) Carstensen *et al*., 1993; l) McFarlane *et al*., 1987; m) Grimmelikhuijzen and Graf, 1986; n) Grimmelikhuijzen and Groeger, 1987; o) Moosler *et al*., 1997: p) Grimmelikhuijzen *et al*., 1988; q) Grimmelikhuijzen *et al*., 1992; r) Moosler *et al*., 1996; s) Leitz *et al*., 1994; t) Takahashi *et al.* 1997; u) Iwao *et al*., 2002; v) Fujisawa, 2008; w) Yum *et al*., 1998; x) Takahashi *et al.,* 2000; Hayakawa *et al*., 2007.

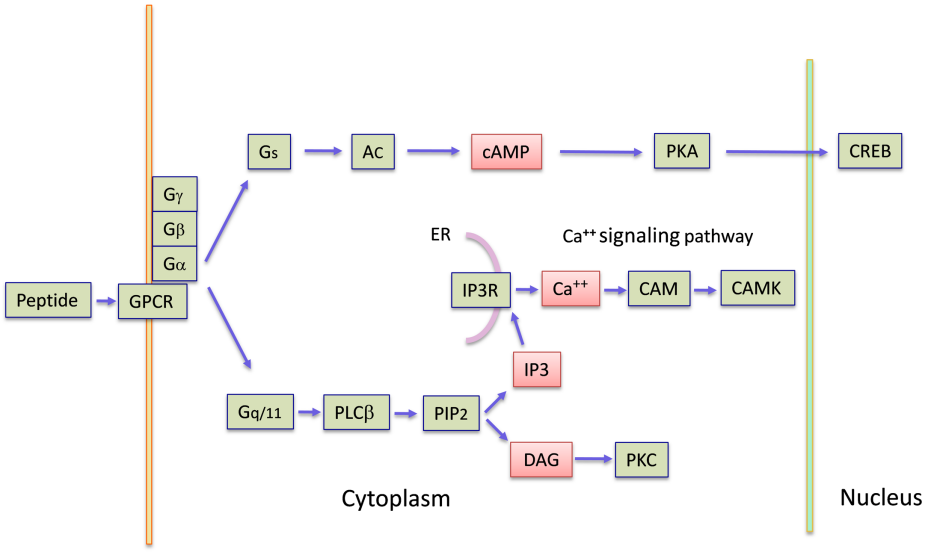

Fig. 1. Peptide - Gprotein coupled receptor (GPCR) signaling pathways.

2001). These peptides contain no modified residues. An amidated peptide with a single intramolecular disulfide bridge, Hym-301 is involved in tentacle formation (Takahashi *et al.*, 2005). As has been pointed out, it is in good agreement with the notion that epithelial cells in *Hydra* are a primary cell type to regulate pattern formation/morphogenesis in *Hydra* (Fujisawa, 2008). Epitheliopeptides with PW residues at the C-terminus inhibit neuron differentiation and counteract against the action of Hym-355 (Takahashi *et al.*, 1997; 2000, 2009). The PW peptides presumably monitor the density of neurons (neurons/epithelial cells) in the body column. If the density of neuron is low, PW peptides do not exert their effect so that the action of Hym-355 could prevail. When the neuron density becomes higher, PW peptides are presumably released extracellularly to counteract against the positive feedback of neuron differentiation by Hym-355. In this way homeostasis of a neuron population is maintained in the *Hydra* body.

There are two remarkable *Hydra* peptides reported to be a neuropeptide (head activator) (Schaller and Bodenmueller, 1981) and an epitheliopeptide (HEADY) (Lohmann and Bosch, 2000). Unfortunately, however, genes encoding these peptides are not found in the *Hydra* genome database (see in this issue Steele, 2012). Thus, the existence of these peptides remains elusive.

Synthesis and release of signaling peptides

Signaling peptides are encoded in a larger precursor protein, which is post-translationally processed via cleavage by specific proteolytic enzymes called prohormone convertases to produce functional units. Processing and cleavage patterns and signal transduction pathways vary dependent on peptide species and cell types. A famous example is proopiomelanocortin, which differentially produces at least 10 different peptide hormones that distribute in the anterior and intermediate lobes of the pituitary gland, hypothalamus and skin (Pritchard and White, 2007).

Neuropeptides: RFamides, RWamides

Most of neuropeptides so far identified in cnidarians are short (around 10 amino acid-long). The precursor protein contains a signal peptide at its N-terminus. The protein is targeted to the endoplasmic reticulum (ER) and the signal peptide is cleaved off to enter the Golgi network. Modification like glycosylation or sulfation at a certain amino acid residue occurs during traversing the Golgi network. Peptides are then packed in secretory vesicles where processing of the protein into peptides and modification at the N-terminus (e.g. acetylation or pyro-glutamate formation) or at the C-terminus (amidation) takes place. Cnidarian neuropeptides have been localized by immunogold-electronmicroscopy. For example, *Hydra* RFamides are localized in the dense-core vesicles of peduncle neurons (Koizumi *et al.* 1989). Antho-RFamides and Antho-RWamides are also localized in excretory vesicles in neurons of anthozoans (Westfall and Grimmelikhuijzen, 1993; Westfall *et al.*, 1995).

These vesicles are directed mainly to the presynaptic membrane where contents of the vesicles are released upon stimulation to the synaptic cleft. The released peptide binds to its receptor on the postsynaptic cells so that the signal could be transmitted to the target cells. However, Antho-RFamides are also detected in non-synaptic vesicles (Westfall and Grimmelikhuijzen, 1993). In the planula larva of the hydrozoan *Pennaria tiarella* the peptide packed-vesicles are found all along the neurite in close contact with the cell membrane (Brumwell and Martin, 1996). Since RFamides inhibit the action of GLWamides that induces metamorphosis of the planula larva of the hydrozoan *Hydractinia echinata* (Katsukura *et al.*, 2003), release of these peptides may occur in the non-synaptic regions as a paracrine factor.

Epitheliopeptides

Precursors with a signal peptide

Hym-301 is so far the only epitheliopeptide with the C-terminal amidation in cnidarians. Since its precursor has a similar structure to the neuropeptide precursors, the peptides are expected to localize in secretory vesicles. Immuno-electronmicroscopy using an anti-Hym-301 antibody revealed that the peptide localize in secretory vesicles called electron-dense inclusions (West, 1978; Wood, 1979) that are located at the apical part of the ectodermal epithelial cells in the head region (Fig. 3; Takaku *et al.*, unpublished results). These vesicles are almost 10 times larger in size (1~1.5 μm in a long axis) comparing to dense-cored vesicles in neurons. The Hym-301 peptide appears to be released outside of cells and also into the cytoplasm.

The precursor of PW peptides is also predicted to have a signal peptide and cleaved at dibasic amino acids at least at the C-terminus of each peptide (Takahashi *et al.*, 2009). However, its subcellular localization is still unknown.

Precursors lacking a signal peptide

Hym-323, Pedin/Hym-330 and Pedibin/Hym346 are involved in foot formation (see Fujisawa, 2008 for review) and all of their precursors lack a signal peptide. Nevertheless, Pedibin/Hym-346 is found secreted extracellularly when tested with the yeast invertase secretion assay (Böttger *et al.*, 2006). Although the mechanism

of its secretion is unknown, some of the proteins without a signal peptide are secreted extracellularly via a chaperon-like protein (Piotrowicz *et al*., 1997; Suzuki *et al*., 2010). Also, a large number of peptides secreted independent of the classical ER-Golgi vesicular pathway have been reported in a large scale peptidomic analysis of mouse brain (Fricker, 2010).

Signal transduction

G-protein coupled receptor (GPCR) signaling

G Protein-coupled receptors (GPCRs) have seven transmembrane domains; the N-terminal region is extracellular, three loops extend each outside and inside the membrane and the C-terminal region is cytoplasmic. There are seven GPCR families that are classified by their structures and functions. The family 1 is rhodopsin-like and the largest group whose ligands are biogenic amines, peptides, glycoprotein hormones, odorants, purines, eicosanoids etc. Signaling peptides generally bind to family 1 GPCRs. Family 2 is secretin-like whose ligands are mostly polypeptide hormones like glucagon, pituitary adenylate cyclase-activating polypeptide (PACAP), vasoactive intestinal polypeptide (VIP) etc. Family 3 includes metabotropic glutamate receptors. Other four families form minor groups. We found 663 family 1 GPCR genes in the *Hydra* genome (Hayakawa and Fujisawa, unpublished). Majority of these GPCR genes appear to be intact. Among the GPCRs, 63 are opsins in *Hydra* (Suga *et al*., 2008).

In *Hydra* there is no known GPCR whose ligand is a peptide, although several GPCRs show significant structural similarities to neuropeptide GPCRs in higher organisms. General scheme for signal transduction of Family 1 GPCR is shown in Fig. 1 (taken from GnRH signaling in KEGG pathway maps, KEGG (http://www.genome.jp/kegg/) and modified). Heterotrimeric G proteins (α, β, γ subunits are coupled with a GPCR. Once a ligand binds to the receptor, GTP replaces GDP that binds to G protein α subunit Gα and the Gα is dissociated from $\beta\gamma$ dimmer (G$\beta\gamma$) to activate enzymes in the signal transduction cascade. There are four subtypes of Gα (Gs, Gq/11, Gi/o and G12/13) classified by their amino acid sequences. Fig. 1 shows three representative pathways that are activated by Gs and Gq/11. Gs activates adenylate cyclase (AC) that produces cAMP as a second messenger, which in turn activates protein kinase A (PKA). PKA is involved in numerous reactions including activation of a transcription factor CREB. Gq/11 activates phospholipase Cβ (PLCβ) which hydrolyses the membrane phospholipid, phosphatidylinositol (4,5)-bisphosphate (PIP2) into two second messengers inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG). IP3 binds to IP3 receptor on the ER inducing Ca^{++} release. Released Ca^{++} may bind to many Ca^{++} binding proteins that mediate variety of biological effects of Ca^{++}. One of them, calmodulin (CaM) activates calcium-calmodulin-dependent protein kinases (CaMKs) that also exert various effects. On the other hand DAG activates protein kinase C (PKC), which activates many cytosolic proteins. The genes encoding all of the proteins described here have their homologues in *Hydra* genome (Chapman *et al*. 2010).

Insulin signaling

The insulin signaling pathway has been characterized in detail in bilateral animals and is responsible for regulations of metabolism, longevity and growth (Taguchi and White, 2008). In *Hydra*, one of the receptor tyrosine kinase genes, *HTK7* encodes a member of insulin receptor family (Steele *et al*., 1996). HTK7 is suggested to regulate growth and patterning. However, its ligand is not known. The search in the *Hydra* ESTs has yielded three genes encoding insulin-like peptides (Nishimiya-Fujisawa and Fujisawa, unpublished; Steele unpublished). One of them is roughly the same size as mammalian insulins, while other two are larger with extended N-terminal regions. Two of the genes can rescue the growth defects in *Drosophila,* in which cells producing insulin-like peptides were ablated (Steele, personal communication). Thus, the functions of the peptides are conserved from Cnidaria to Arthropoda, although in vivo functions in *Hydra* remain to be discovered. The insulin signaling pathway obtained from higher metazoans is shown in Fig. 2 (taken from insulin signaling in KEGG pathway maps, KEGG (http://www.genome.jp/kegg/) and modified).

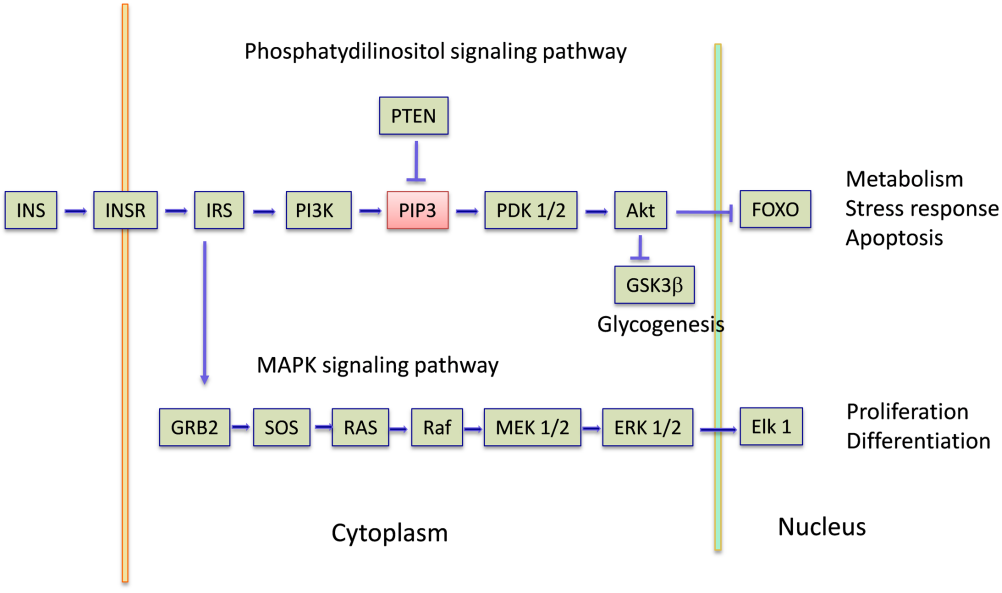

Fig. 2. Insulin signaling pathways.

Insulin binds to an insulin receptor which is composed of two α subunits and two β subunits. The insulin receptor phosphorylates insulin receptor substrates (IRSs). In the phosphatidylinositol signaling pathway, the IRS binds to PI3K, which phosphorylates phosphatidylinositol (4,5)-bisphosphate (PIP2) to produce a second messenger, phosphatidylinositol (3,4,5)-trisphosphate (PIP3). PIP3 is negatively regulated by phosphatase and tensin homolog (PTEN). PIP3 activates 3-phosphoinositide-dependent kinase, PDK1/2 that in turn phosphorylates serine/threonine kinase, Akt. Activated Akt phosphorylates, thus inhibits a fork-head transcription factor, FOXO by promoting its nuclear export and glycogen synthase kinase (GSK)

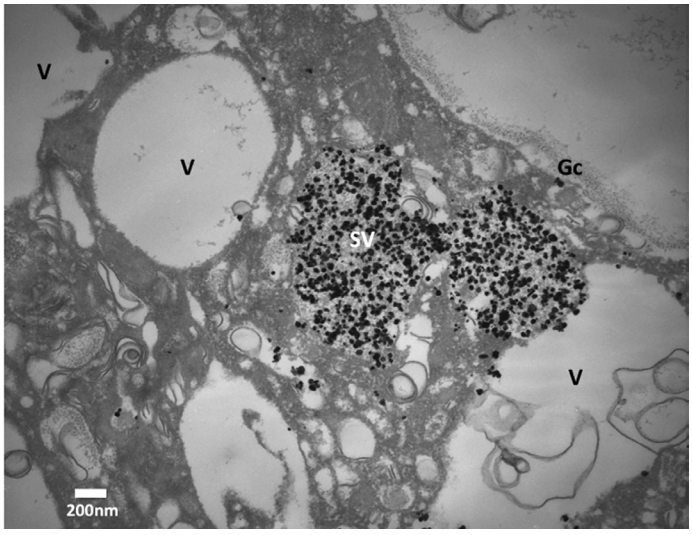

Fig. 3. Immuno-electron microscopy using an anti-Hym-301 antibody. *Notice the numerous gold particles in the secretory vesicles (SV) located in the ectodermal epithelial cell. Gc, glycocalyx; V, vacuole.*

3β. In the MAPK signaling pathway, IRS associates with growth factor receptor-bound protein, GRB-2. The complex activates Son of Sevenless (SOS) by removing GDP from and adding GTP to Ras, which then pushes the MAPK cascade down with sequential phosphorylation. Elk1 is a transcription factor that activates genes involved in cell proliferation and differentiation. The MAPK cascade and CREB signaling in regeneration have been reported in *Hydra* (Galliot *et al*., 1995; Kaloulis *et al*., 2004; Chera *et al*., 2007; Chera *et al*., 2011). All of the genes encoding the proteins described here except for Elk1 have their homologues in *Hydra* genome (Galliot *et al*., 1995; Fujisawa, unpublished). ERK 1/2 might activate other transcription factors like c-myc or other ETS domain containing transcription factors.

Evolutionary considerations concerning peptides, receptors and channels

Peptides

Peptides described in this review are rather short and the motif essential for the function is even shorter (two or three amino acid-long). Thus, it is generally difficult to discuss the evolutionary conservation of peptides. Only short peptides conserved among cnidarians are RFamide and GLWamide families. When many peptides with the same motif are encoded in a precursor protein, it is relatively easy to find homologs based on the conserved motif among other animals even in different phyla. RFamides are such an example. RFamides appear to be conserved from cnidarians to mammals and some of the functions like cardiac muscle contraction (or pumping) can be seen from *Hydra* to man (Shimizu and Fujisawa, 2003).

GLWamides are easily found among cnidarians with the same approach as for RFamides. But, when it comes to other phyla, it becomes difficult to find homologs by conventional searches such as BLAST. Instead one has to search by considering the conserved structural hallmarks, e.g. presence of a signal peptide, dibasic cleavage sites, amidation motifs and conserved motifs in mature peptide sequences. By doing so, we identified a GLWamide precursor protein in *C. elegans* (Ishihara *et al*., unpublished data, 1998; Fujisawa, 2008) and later this was confirmed biochemically (Husson *et al*., 2005). Homologs of GLWamide genes have not been detected in other metazoans. Thus, it is apparent that RFamides and GLWamides were acquired in the first nervous system of cnidarian ancestors and not only the amino acid motifs but also their basic functions appear to be maintained in extant metazoans.

It is conceivable that orthologs of other peptide families may be found in animals in different phyla. However other peptide genes such as *Hym-176* and *Hym-301* or their related genes are not found outside of *Hydra*. These paralogs, although related, have rather diverse sequences. Thus, an ancestral gene was presumably acquired after *Hydra* branched from other hydrozoans and later diverged to perform related but different functions. These genes are referred to as taxonomically restricted genes that play a role in the creation of phylum (or even family)-specific novelties (Milde *et al*., 2009).

G-protein coupled receptors

As mentioned above the signaling pathway of GPCR appears to be well conserved from *Hydra* to mammals indicating that the whole pathway is important for animal survival. The number of GPCRs per genome generally increases as the phylogenetic order goes up and this increase is mainly attributed to the expansion of odorant or chemosensory receptors (Table 2). A unicellular eukaryote, *Tetrahymena* has only several GPCRs (Lampert *et al*., 2011) while slime mold, *Dictyostelium discoideum* has 55 genes for GPCR (Prabhu *et al*., 2007). One of the most primitive metazoan, sponge has more than 200 GPCRs. The sudden increase in the number of GPCRs appears to occur to attain multicellularity of animals. *C. elegans* has 1006 odorant/chemosensory receptors among 1149 GPCRs (Table 2; see Frederiksson *et al*., 2005).

Unexpectedly *Hydra* genome contains 822 GPCRs, 663 related to family-1, 41 to family-2 and 118 to family-3 (Hayakawa and Fujisawa, unpublished observation), *Nematostella* about 500. As in case of vertebrates but not of *C. elegans* and *Drosophila*, most of the *Hydra* GPCR genes are single exon genes. They are arrayed in tandem along the contigs. Also, most of them appear to

TABLE 2

NUMBER OF GPCR GENES IN REPRESENTATIVE EUKARYOTE GENOMES

Organism(s)	Number	References
Yeasts	3-9	Frederiksson and Schio, 2005 [1)]
Plants	1-6	Frederiksson and Schio, 2005 [1)]
Slime mold (*Dictyostelium*)	55	Prabhu *et al*., 2007
Tetrahymena	Several	Lampert *et al*., 2011
Sponge (*Amphimedon*)	>200	Srivastava, *et al*., 2010
Hydra magnipapillata	822	Hayakawa *et al*., unpublished
Sea anemone (*Nematostella*)	~500	Hayakawa *et al*., unpublished
Nematode (*C. elegans*)	1149	Frederiksson and Schio, 2005 [1)]
Odorant receptors	1006/1149	Frederiksson and Schio, 2005 [1)]
Fruit fly (*D. melanogaster*)	210	Frederiksson and Schio, 2005 [1)]
Tunicate (*Ciona*)	208	Frederiksson and Schio, 2005 [1)]
Zebrafish (*Danio*)	1318	Frederiksson and Schio, 2005 [1)]
Mouse (*Mus)*	737	Frederiksson and Schio, 2005 [1)]
Human (*Homo*)	865	Frederiksson and Schio, 2005 [1)]

1) Review article

be intact. The reason for this abundance of GPCRs in cnidarians is not known. One explanation might be that at least *Hydra* uses a few hundreds of peptides as signaling molecules and thus the receptors for these ligands are numerous. The other possibility is that cnidarians uses many chemosensory receptors to monitor environmental cues although there are no good reasons that cnidarians should have so many chemosensory receptors. In any case, the genes with a single exon suggest that the burst of GPCR gene expansion presumably occurred recently in the lineage of *Hydra* evolution since *Hydra* diverged from anthozoans at least 500 millions year ago (Chapman *et al*., 2010).

RFamide-gated ion channels (FaNaCs)

As mentioned above, receptors for peptides are in most cases GPCRs. To date, only exception to this rule is the FMRFamide-gated Na^+ channel first identified in snail *Helix asperosa* (Cotrell *et al*., 1990; Lingueglia *et al*, 1995). FaNaC is a member of the degenerin/epithelial Na^+ channel (DEG/ENaC) family that also includes acid-sensing ion channels (ASICs) (Lingueglia, 2007) (see also in this issue the review on ligand-gated ion channels by Pierobon). The channels in this family are activated by ligands (ASICs) and mechanical forces (degenerins) or opened constitutively (ENaCs) but are blocked by a diuretic drug amiloride. The channel has two transmembrane domains with a long extracellular loop with a cysteine-rich region and N-terminal and C-terminal cytoplasmic domains (Kellenberger and Schild, 2002). *Hydra* contains 4 subunits for FaNaCs designated to as HyNaC 2, 3, 4 and 5. HyNaC2, 3 and 5 form an ion channel gated by *Hydra* RFamide I and II (Golubovic *et al*., 2007; Dürrnagel *et al*., 2010). HyNaC 4 might form the channel with yet un-identified subunits.

Whole mount in situ hybridization shows that the genes encoding these subunits are all expressed in most likely epitheliomuscular cells at the base of the tentacles with a subtle but interesting difference (Dürrnagel *et al*., 2010). The precise in vivo function of this channel in *Hydra* is unknown. However, since the regions expressing these genes and the gene encoding RFamides I and II roughly coincide, the channel may mediate fast axonal transmission to muscle cells at the base of the tentacles to regulate tentacle movement. Recent finding also suggest its involvement in feeding response because amiloride delayed feeding response evoked by glutathione (Dürrnagel *et al*., 2010). This type of channel has been found only in Cnidaria and Mollusca and therefore its evolutionary pathway is unclear. The absence of the channel in other phyla may be a gene loss or due to a technical problem to detect it. Independent incidence to acquire the channels in different phyla is highly unlikely because the same type of neuropeptide gates the channels.

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