REVIEW

The evolution and nomenclature of GnRH-type and corazonin-type neuropeptide signaling systems

Meet Zandawala[1], Shi Tian[2] and Maurice R. Elphick[2*]

1. Stockholm University, Department of Zoology, Stockholm, Sweden

2. Queen Mary University of London, School of Biological & Chemical Sciences, Mile End Road, London, E1 4NS, UK

* Corresponding author: m.r.elphick@qmul.ac.uk

Tel: +44(0) 20 7882 6664

Fax: +44(0) 20 7882 7732

ABSTRACT

Gonadotropin-releasing hormone (GnRH) was first discovered in mammals on account of its effect in triggering pituitary release of gonadotropins and the importance of this discovery was recognized forty years ago in the award of the 1977 Nobel Prize for Physiology or Medicine. Investigation of the evolution of GnRH revealed that GnRH-type signaling systems occur throughout the chordates, including agnathans (e.g. lampreys) and urochordates (e.g. sea squirts). Furthermore, the discovery that adipokinetic hormone (AKH) is the ligand for a GnRH-type receptor in the arthropod *Drosophila melanogaster* provided evidence of the antiquity of GnRH-type signaling. However, the occurrence of other AKH-like peptides in arthropods, which include corazonin and AKH/corazonin-related peptide (ACP), has complicated efforts to reconstruct the evolutionary history of this family of related neuropeptides. Genome/transcriptome sequencing has revealed that both GnRH-type receptors and corazonin-type receptors occur in lophotrochozoan protostomes (annelids, mollusks) and in deuterostomian invertebrates (cephalochordates, hemichordates, echinoderms). Furthermore, peptides that act as ligands for GnRH-type and corazonin-type receptors have been identified in mollusks. However, what has been lacking is experimental evidence that distinct GnRH-type and corazonin-type peptide-receptor signalling pathways occur in deuterostomes. Importantly, we recently reported the identification of two neuropeptides that act as ligands for either a GnRH-type receptor or a corazonin-type receptor in an echinoderm species – the common European starfish *Asterias rubens*. Discovery of distinct GnRH-type and corazonin-type signaling pathways in this deuterostomian invertebrate has demonstrated for the first time that the evolutionarily origin of these paralogous systems can be traced to the common ancestor of protostomes and deuterostomes. Furthermore, lineage-specific losses of corazonin signaling (in vertebrates, urochordates and nematodes) and duplication of the GnRH signaling system in arthropods (giving rise to the AKH and ACP signaling systems) and quadruplication of the GnRH signaling system in vertebrates (followed by lineage-specific losses or duplications) accounts for the phylogenetic distribution of GnRH/corazonin-type peptide-receptor pathways in extant animals.

Informed by these new insights, here we review the history of research on the evolution of GnRH/corazonin-type neuropeptide signaling. Furthermore, we propose a standardized nomenclature for GnRH/corazonin-type neuropeptides wherein peptides are either named "GnRH" or "corazonin", with the exception of the paralogous GnRH-type peptides that have arisen by gene duplication in the arthropod lineage and which are referred to as "AKH" (or red pigment concentrating hormone, "RCPH", in crustaceans) and "ACP".

KEY WORDS

Gonadotropin-releasing hormone; corazonin; adipokinetic hormone; AKH/corazonin-related peptide; red pigment concentrating hormone; evolution; neuropeptide; receptor

1. Introduction

In 1971 a hypothalamic neuropeptide that triggers pituitary release of gonadotropins was identified and named gonadotropin-releasing hormone (GnRH; pQHWSYGLRPGamide) (Amoss et al., 1971; Schally et al., 1971). The importance of this landmark discovery in the field of neuroendocrinology was recognized in the award of the 1977 Nobel Prize for Physiology or Medicine to Roger Guillemin and Andrew Schally. Less well known is that in the six-year period from the discovery of GnRH in 1971 to the Nobel award in 1977, two neuropeptides were identified in invertebrate species that we now know are homologs of GnRH – the crustacean neuropeptide red pigment concentrating hormone (RPCH), which was identified in 1972 (Fernlund and Josefsson, 1972), and the insect neuropeptide adipokinetic hormone (AKH), which was identified in 1976 (Stone et al., 1976). RPCH (pQLNFSPGWamide) and AKH (pQLNFTPNWGTamide) are structurally very similar, clearly indicating that they are evolutionarily related. However, the relationship of RPCH and AKH with GnRH was not apparent at the time of their discovery because of a low level of sequence similarity.

In 1984 the gene encoding the precursor of human GnRH was sequenced, revealing that GnRH is derived from a ninety-two residue protein in which the GnRH is located immediately after the N-terminal signal peptide (Seeburg and Adelman, 1984). In 1992 the first GnRH receptor to be sequenced was discovered in mice (Reinhart et al., 1992; Tsutsumi et al., 1992) and then in 1998 a *Drosophila* homolog of this receptor was cloned and sequenced (Hauser et al., 1998). Four years later the ligand for the *Drosophila* GnRH-type receptor was identified as AKH (Staubli et al., 2002) and thus a hitherto unknown homologous relationship between AKH and GnRH was discovered.

AKH, RPCH and GnRH are members of a family of neuropeptides that occur throughout the Bilateria (Jekely, 2013; Mirabeau and Joly, 2013). However, reconstructing the evolutionarily history of this neuropeptide family has been complicated by the discovery in insects of other neuropeptides that share sequence similarity with AKH. Thus, in 1989 an AKH-like peptide was identified in the cockroach *Periplaneta americana* that has a cardioacceleratory effect and which

was named corazonin ("corazon" is the Spanish word for 'heart') (Veenstra, 1989). Subsequently, this peptide was isolated independently from locusts using ELISA (Veenstra, 1991) and on account of its ability to trigger dark pigmentation (Tawfik et al., 1999). Then in 1999 another AKH-like peptide was identified in locusts (Siegert, 1999) and when the receptor for this peptide, which is distinct from yet closely related to AKH receptors and corazonin receptors, was discovered in the mosquito *Anopheles gambiae*, the peptide was named AKH/corazonin-related peptide or ACP (Hansen et al., 2010).

Insights into the evolution of GnRH/AKH/ACP/corazonin-type neuropeptide signaling have been obtained from other invertebrates. For example, GnRH/AKH/ACP/corazonin-like peptides have been identified in nematodes (Lindemans et al., 2009) and mollusks (Iwakoshi et al., 2002; Johnson et al., 2014) and a putative ligand for a GnRH/corazonin-type receptor has been identified in a deuterostomian invertebrate, the cephalochordate *Branchiostoma floridae* (Roch et al., 2014b). Furthermore, a key breakthrough in our understanding of the evolution of GnRH/AKH/ACP/corazonin-type neuropeptides was made recently with the discovery of distinct GnRH-type and corazonin-type neuropeptide signaling pathways in another deuterostomian invertebrate – the starfish *Asterias rubens* (Phylum Echinodermata) (Tian et al., 2016). Importantly, this finding revealed that the evolutionary origin of paralogous GnRH-type and corazonin-type neuropeptide signaling pathways can be traced back at least as far as the common ancestor of protostomes and deuterostomes. In light of this discovery, a re-evaluation of previously published literature and nomenclature in this field of research is necessary and timely.

In the first part of this review we describe some of the key discoveries that led to our current understanding of the evolution of GnRH/corazonin-type neuropeptide signaling systems (Fig. 1). In the second part of the review we discuss how our recent findings provide a basis for establishing a standardized nomenclature for GnRH/corazonin-type neuropeptides, highlighting the need for a standardized nomenclature with reference to selected publications.

2. Key discoveries in the history of research on the evolution of GnRH-type and corazonin-type neuropeptide signaling.

2.1. The evolution of GnRH signaling in vertebrates: discovery of the structure and functions of GnRH-type peptides in agnathans

In the decade or so following the identification of GnRH in mammals, the occurrence and actions of GnRH in other vertebrates was investigated (Peter et al., 1987). We now know that there are multiple genes encoding GnRH-type peptides and receptors in most vertebrate species, which reflects the occurrence of genome duplications during vertebrate evolution (Yun et al., 2015). Furthermore, there have been subsequent losses of these paralogs in multiple vertebrate lineages (Roch et al., 2014a). A detailed discussion of the evolution and diversification of GnRH signaling in vertebrates is beyond the scope of this review, and this topic has been ably reviewed previously (Okubo and Nagahama, 2008; Kim et al., 2011; Roch et al., 2011; Sower et al., 2012; Decatur et al., 2013; Roch et al., 2014b). However, it is appropriate in a special issue of *General & Comparative Endocrinology* dedicated to Stacia Sower to highlight here her research on GnRH signaling in agnathan vertebrates.

Purification and sequencing of a GnRH-type peptide from an agnathan, the lamprey *Petromyzon marinus*, provided the first definitive evidence of the antiquity of GnRH in the vertebrate lineage (Sherwood et al., 1986). Furthermore, the identification of this lamprey GnRH enabled Stacia Sower and colleagues to investigate the physiological roles of the native peptide in agnathans. An ancient role in regulation of reproductive physiology was revealed with the discovery that lamprey GnRH stimulates the pituitary-gonadal axis in adult male lampreys as determined by steroidogenesis and spermiation (Sower, 1989). Subsequently, multiple GnRH-type neuropeptides and receptors have been identified in lampreys (Sower et al., 1993; Silver et al.,

2004; Silver et al., 2005; Kavanaugh et al., 2008; Joseph et al., 2012; Osugi et al., 2012; Freamat and Sower, 2013).

2.2. Discovery of GnRH-type neuropeptides in urochordates

Indirect evidence for the occurrence of GnRH-like peptides in invertebrates was first obtained through the use of immunocytochemistry, employing antibodies to mammalian GnRH (Dubois, 1980; Georges and Dubois, 1980). Furthermore, pharmacological effects of mammalian GnRH on invertebrate preparations were also reported (Steiner and Felix, 1989). However, the first definitive molecular evidence that GnRH-type neuropeptides occur in invertebrates was the sequencing of two GnRH-like peptides isolated from an invertebrate chordate, the urochordate *Chelyosoma productum*. Furthermore, the presence of GnRH-immunoreactive neurons located within blood sinuses close to the gonoducts and gonads in *Chelyosoma* was considered indicative of a role in which GnRH-type peptides act directly on the gonads (Powell et al., 1996). Subsequently, sequencing of the genome of the urochordate *Ciona intestinalis* enabled detailed characterization of GnRH-type signaling systems comprising multiple peptides and receptors (Adams et al., 2003; Kusakabe et al., 2003). Interestingly, evidence of both reproductive and non-reproductive functions of GnRH-type signaling systems in *Ciona* has been obtained (Terakado, 2001; Kusakabe et al., 2012; Kamiya et al., 2014).

2.3. Discovery of AKH, corazonin and AKH/corazonin-related peptide (ACP) neuropeptide-receptor pathways in insects reveals a relationship with GnRH signaling

The first GnRH-type peptide to be isolated from an invertebrate and sequenced was the crustacean hormone RPCH (Fernlund and Josefsson, 1972), but its relationship with GnRH was not recognised at the time of its discovery. RPCH was purified from eyestalks of the prawn *Pandalus*

borealis on account of its ability to stimulate changes in body coloration due to pigment migration in chromatophores (Fernlund and Josefsson, 1968; Fernlund and Josefsson, 1972). Soon after this discovery, an RPCH-like peptide was isolated from the corpora cardiaca (CC) of the locust *Schistocerca gregaria* on account of its ability to mobilize lipids from fat bodies, and hence it was named adipokinetic hormone or AKH (Mayer and Candy, 1969; Stone et al., 1976).

Over the next decade or so, several members of the AKH/RPCH peptide family were isolated from various arthropods (Scarborough et al., 1984; Ziegler et al., 1985; Jaffe et al., 1986; Gade, 2009), with their expression patterns and actions examined using immunocytochemistry (Schooneveld et al., 1983; Schooneveld et al., 1985; 1987a; Schooneveld et al., 1987b; Clottens et al., 1989) and various bioassays (Mordue and Stone, 1977; Shapiro and Law, 1983; Goldsworthy et al., 1986; Goldsworthy and Wheeler, 1986), respectively. The first AKH-type peptide precursor gene to be sequenced was identified in the locust *Schistocerca gregaria* (Schulz-Aellen et al., 1989), revealing that the AKH peptide is located immediately after the N-terminal signal peptide, as seen in GnRH precursors. However, at this time in 1989 this structural similarity between the locust AKH precursor and GnRH-type precursors was not recognized. At the same time an AKH-like peptide with cardioacceleratory properties isolated from the cockroach *Periplaneta americana* was identified as the prototype for a novel neuropeptide family – the corazonins (Veenstra, 1989). Furthermore, sequencing of the *Drosophila* gene encoding the corazonin (or CRZ) precursor revealed similarities with the AKH precursor, providing additional evidence that AKH and corazonin are evolutionarily related (Veenstra, 1994).

The first evidence of a GnRH-like signaling system in insects was the sequencing of a *Drosophila* G-protein coupled receptor related to vertebrate GnRH-type receptors (Hauser et al., 1998). Then in 2002, the ligand of the GnRH-type receptor in *Drosophila* and in the silkworm *Bombyx mori* was identified as AKH (Park et al., 2002; Staubli et al., 2002). This was a major breakthrough in arthropod neuroendocrinology because up until that point AKH/RPCH-type peptides were thought of as members of an arthropod-specific neuropeptide family. Thus, the

discovery of insect AKH receptors united GnRH in chordates and AKH/RPCH in arthropods as a single bilaterian neuropeptide family. The functional characterization of the corazonin receptor from *Drosophila* in the same year provided the first insights into the evolutionary relationships of corazonin, AKH and GnRH signaling systems (Cazzamali et al., 2002; Park et al., 2002). Thus, phylogenetic analysis suggested that AKH receptors and GnRH receptors may be orthologous, with corazonin receptors a closely-related outgroup. However, the support for this hypothesis was based on low bootstrap values and the precise evolutionary origins of these signaling systems remained unresolved.

The discovery of the *Drosophila* and *Bombyx* AKH receptors and the *Drosophila* corazonin receptor paved the way for functional characterization of these receptors in other insects (Kim et al., 2004; Belmont et al., 2006; Hansen et al., 2006; Zhu et al., 2009; Huang et al., 2011; Konuma et al., 2012; Yang et al., 2013; Zandawala et al., 2015b; Hamoudi et al., 2016). With the identification of the AKH receptor and the corazonin receptor in the mosquito *Anopheles gambiae*, it became evident that there exists a signaling system closely related to the AKH system (Belmont et al., 2006). Phylogenetic analysis revealed that *Anopheles* has another receptor that is closed related to the AKH receptor but which is absent in *Drosophila* and which is not activated by *Anopheles* AKH. Belmont et al. (2006) suggested that perhaps this "orphan" receptor is activated not by AKH but by other AKH-like peptides, which had been found previously in locusts (Karl et al., 1985; Oudejans et al., 1991; Siegert, 1999; Belmont et al., 2006) This speculation was proven correct in 2010 when the endogenous ligand for this receptor was found to be an ortholog of a locust AKH-like peptide that lacks adipokinetic activity (no lipid mobilizing effect) and related AKH-like peptides in mosquitoes (Siegert, 1999; Kaufmann and Brown, 2006; Kaufmann et al., 2009; Hansen et al., 2010). This novel neuropeptide type was termed AKH/corazonin-related peptide (ACP) on account of its sequence similarity with both AKH and corazonin. More recent studies have revealed that the AKH and ACP signaling systems are paralogous, having arisen by a gene duplication in the arthropod lineage (Hauser and Grimmelikhuijzen, 2014). However, following this duplication, the

ACP signaling system has been lost independently in several arthropods, including *Drosophila*, the aphid *Acyrthosiphon pisum* and the crustacean *Daphnia pulex* (Hansen et al., 2010).

Functional characterization of the AKH and corazonin signaling systems has been facilitated by the availability of a plethora of molecular and genetic tools in *Drosophila*. For instance, it has been discovered that AKH regulates energy/nutrient homeostasis (Kim et al., 2004; Galikova et al., 2015; Hentze et al., 2015; Sajwan et al., 2015), nutritional and oxidative stress (Bharucha et al., 2008; Bednarova et al., 2015; Zemanova et al., 2016), hunger (Jourjine et al., 2016), starvation-induced hyperactivity (Lee and Park, 2004; Isabel et al., 2005; Yu et al., 2016), and lifespan (Waterson et al., 2014), whereas corazonin regulates nutritional and oxidative stress (Zhao et al., 2010; Kubrak et al., 2016), feeding (Kubrak et al., 2016), nutrient-sensing (Miyamoto and Amrein, 2014), ethanol-related behavior and metabolism (McClure and Heberlein, 2013; Sha et al., 2014; Varga et al., 2016), sperm transfer and copulation (Tayler et al., 2012), and fecundity (Bergland et al., 2012). The absence of ACP in *Drosophila* has limited the number of studies examining its physiological roles, which remain unknown (Hansen et al., 2010; Zandawala et al., 2015a). Nonetheless, the data that are available suggest functions distinct from those of AKH and corazonin (Siegert, 1999; Patel et al., 2014).

2.4. Phylogenetic analysis of GnRH/AKH/ACP/corazonin-type signaling systems

Recent advances in genomics/transcriptomics have enabled comprehensive investigation of the occurrence of neuropeptide signaling systems in a wide range of phyla (Veenstra, 2010; 2011; Conzelmann et al., 2013a; Jekely, 2013; Mirabeau and Joly, 2013; Semmens et al., 2016). These include lophotrochozoan protostomes (annelids and mollusks) and ambulacrarians (echinoderms and hemichordates), which occupy an intermediate phylogenetic position between chordates (including vertebrates) and protostomes. Accordingly, deorphanisation of G-protein coupled receptors in lophotrochozoans and ambulacrarians has provided important new insights into the

239 evolution of neuropeptide signaling systems (Conzelmann et al., 2013b; Bauknecht and Jekely,
240 2015; Semmens et al., 2015; Semmens et al., 2016).
241 Several studies have investigated the phylogenetic distribution and evolutionary origins of
242 the GnRH, AKH, ACP and corazonin signaling systems, utilizing transcriptome/genome sequence
243 data from species belonging to a wide range of phyla (Roch et al., 2011; Jekely, 2013; Mirabeau
244 and Joly, 2013; Hauser and Grimmelikhuijzen, 2014; Roch et al., 2014a; Roch et al., 2014b;
245 Plachetzki et al., 2016). Roch et al. (2011) showed that corazonin receptor-type proteins are not
246 only found in arthropods but are also present in other protostomes (mollusks, annelids) and in
247 invertebrate deuterostomes (hemichordates, cephalochordates) (Roch et al., 2011). These authors
248 referred to these receptors as corazonin/GnRH receptors and concluded that "a single ancestral
249 receptor duplicated to produce the basal corazonin/GnRH receptor and the GnRH/AKH receptor
250 ancestor. Most lineages have retained their corazonin/GnRH receptor paralog, except for the
251 nematodes, tunicates and vertebrates." Similarly, Mirabeau and Joly (2013) showed that
252 GnRH/AKH-type receptors and corazonin-type receptors form distinct clades, with protostomian
253 and deuterostomian representation in both clades, but they classified these as a single bilaterian
254 GnRH/corazonin signaling system (Mirabeau and Joly, 2013). Collectively, the data reported by
255 Roch et al. (2011) and Mirabeau and Joly (2013) are consistent with the notion that distinct
256 GnRH/AKH/ACP-type and corazonin-type signaling systems originated by duplication of an
257 ancestral signaling system in a common ancestor of the protostomes and the deuterostomes (Roch et
258 al., 2011; Mirabeau and Joly, 2013). However, the molecular identity of the peptides that act as
259 ligands for corazonin-type receptors in deuterostomes was, until recently, unknown.
260 Tello and Sherwood (Tello and Sherwood, 2009) and Roch et al. (Roch et al., 2011; Roch et
261 al., 2014b) investigated the ligand-binding properties of GnRH/corazonin-type receptors in the
262 cephalochordate *Branchiostoma floridae* (amphioxus). Phylogenetic analysis revealed that there are
263 four GnRH/corazonin-type receptors in this species: two of these receptors (amphioxus GnRHR1
264 and GnRHR2) are orthologs of vertebrate GnRH receptors and arthropod AKH/ACP receptors and

265 the other two receptors (amphioxus GnRHR3 and amphioxus GnRHR4) are orthologs of a group of
266 receptors that include arthropod corazonin receptors and mollusk/annelid receptors that are
267 described as "invertebrate GnRH receptors" (Roch et al., 2011). In an effort to characterize the
268 ligand-binding properties of three of these receptors, Tello and Sherwood (2009) tested vertebrate
269 GnRH1 and GnRH2, an octopus GnRH-like peptide (which we now know is the ligand for a
270 corazonin-type receptor, see below) and an insect AKH as ligands (Tello and Sherwood, 2009).
271 Amphioxus GnRHR1 and GnRHR2 were activated by the vertebrate GnRHs but not by the
272 invertebrate peptides. Amphioxus GnRHR3 was activated by all four peptides but the two
273 invertebrate peptides were more potent that the vertebrate peptides. These data provided important
274 pharmacological evidence that, consistent with phylogenetic sequence analysis, the amphioxus
275 receptors are GnRH/corazonin-type receptors. However, identification of the endogenous ligands
276 for these receptors was required for a definitive characterization of their pharmacological
277 properties.
278 Importantly, Roch et al. (2014) identified a gene in *B. floridae* that encodes the precursor of
279 a putative GnRH-like peptide – pQILCARAFTYTHTWamide (Roch et al., 2014b). This peptide
280 does not act as a ligand for the amphioxus receptors GnRHR1, 2 and 4, and thus the ligands for
281 these receptors remain unknown. The amphioxus GnRH-like peptide does, however, act as a ligand
282 for amphioxus GnRHR3, although its potency was found not to be significantly higher than the
283 octopus GnRH-like peptide - pQNYHFSNGWHPGamide. Our analysis of the sequence of the *B.*
284 *floridae* GnRH-like peptide precursor has revealed the presence of a predicted signal peptide
285 cleavage site between alanine and phenylalanine residues - pQILCARAFTYTHTWamide (Tian et
286 al., 2016). Furthermore, the presence of a single cysteine residue in this putative GnRH-like peptide
287 would imply that, if produced, this peptide would exist as a dimer. Therefore, we propose that the
288 peptide derived from the *B. floridae* GnRH-like peptide precursor is FTYTHTWamide, a truncated
289 form of the peptide predicted by Roch *et al.* (Roch et al., 2014b). If this hypothesis is correct, it may
290 explain why the longer peptide tested by Roch et al. (pQILCARAFTYTHTWamide) had relatively

low potency as a putative endogenous agonist (Roch et al., 2014b). Further experiments are now needed to determine if FTYTHTWamide or pQILCARAFTYTHTWamide are present in extracts of *B. floridae* and to investigate if FTYTHTWamide is more or less potent than pQILCARAFTYTHTWamide as an agonist for amphioxus GnRHR3. In addition, it would be interesting to test FTYTHTWamide as a candidate agonist for amphioxus GnRHR4, which is closely related to amphioxus GnRHR3.

2.5. Discovery of distinct GnRH-type and corazonin-type neuropeptide-receptor pathways in an echinoderm

Analysis of genome/transcriptome sequence data from the sea urchin *Strongylocentrotus purpuratus* (Phylum Echinodermata) revealed the presence of three GnRH-type receptors (Roch et al., 2011; Tian et al., 2016) and a single corazonin-type receptor (Roch et al., 2014b; Tian et al., 2016). However, only a single GnRH-type peptide precursor was identified in *S. purpuratus* (Roch et al., 2011; Rowe and Elphick, 2012). More recently, analysis of transcriptome sequence data from another echinoderm species, the common European starfish *Asterias rubens*, revealed that there are in fact two precursors of GnRH-like peptides in both *A. rubens* and *S. purpuratus* (Semmens et al., 2016; Tian et al., 2016). Furthermore, analysis of *A. rubens* transcriptome data identified two GnRH/corazonin-type receptors in this species. A comprehensive phylogenetic analysis of GnRH-type receptors and corazonin-type receptors from several phyla revealed that one of the starfish receptors groups with GnRH/AKH/ACP receptors, including amphioxus GnRHR1, 2, and therefore we named this receptor *A. rubens* GnRHR or ArGnRHR. The other starfish receptor groups with protostomian corazonin-type receptors and amphioxus GnRHR3, 4, and therefore we named this receptor *A. rubens* CRZR or ArCRZR (Fig. 2) (Tian et al., 2016).

Having identified ArGnRHR and ArCRZR as well as two precursors of GnRH-like peptides in *A. rubens*, we set out to determine if these are ligand-receptor partners. Following determination of the structures of the two GnRH-like peptides by mass spectrometric analysis of nerve extracts

317 from *A. rubens*, the peptides were tested as ligands for ArGnRHR and ArCRZR in a heterologous
318 cellular assay. We discovered that the *A. rubens* GnRH-like peptide pQIHYKNPGWGPGamide is a
319 potent agonist for ArGnRHR, but has no activity as a ligand for ArCRZR; hence we named this
320 peptide *A. rubens* GnRH or ArGnRH. Conversely, we found that the second *A. rubens* peptide
321 HNTFTMGGQNRWKAGamide is a potent agonist for ArCRZR, but has no activity as a ligand for
322 ArGnRHR; hence we named this peptide *A. rubens* corazonin or ArCRZ (Tian et al., 2016).
323 Importantly, ArCRZ is the first ligand for a corazonin-type receptor to be biochemically identified
324 in a deuterostomian invertebrate. Furthermore, our discovery of distinct GnRH-type and corazonin-
325 type signaling pathways in a deuterostomian invertebrate, the starfish *A. rubens*, provided important
326 new evidence that these paralogous signaling systems originated by gene duplication in a common
327 ancestor of protostomes and deuterostomes. The GnRH signaling system appears to have been
328 retained in the majority of animal phyla, with a second duplication of the GnRH system giving rise
329 to the AKH/ACP systems in arthropods. In contrast, the corazonin signaling system has been lost in
330 multiple lineages, including vertebrates, urochordates, nematodes and some insects (e.g.
331 Coleoptera) (Fig. 3).
332 The proposed gene duplication events in a common ancestor of protostomes and
333 deuterostomes that gave rise to the GnRH-type and corazonin-type signaling systems are reflected
334 by gene synteny in extant animals. Thus, comparison of genome sequence data from vertebrates, the
335 cephalochordate *B. floridae* and the mollusk *Lottia gigantea* reveals synteny of GnRH-type receptor
336 genes and corazonin-type receptor ("InvGnRH receptor") genes, but with loss of corazonin-type
337 receptor genes in vertebrates (Roch et al., 2014a). As genome sequence data becomes available for
338 species from a wider range of phyla, it may be possible to gain further insights into the evolution of
339 GnRH/corazonin signaling from analysis of gene synteny.
340 Interestingly, comparative analysis of the sequences of the starfish peptides ArGnRH and
341 ArCRZ with GnRH/AKH/ACP/corazonin-type peptides in other phyla revealed that there do not
342 appear to be any structural characteristics that uniquely distinguish GnRH/AKH/ACP-type peptides

on the one hand and corazonin-type peptides on the other (Tian et al., 2016). Likewise, analysis of gene structure (i.e. positions of introns and/or intron phasing) do not reveal features that universally distinguish GnRH/AKH/ACP-type precursor genes from corazonin-type precursor genes or that distinguish GnRH/AKH/ACP-type receptor genes from corazonin-type receptor genes (Roch et al., 2014b; Semmens et al., 2016; Tian et al., 2016). One possible explanation for this may be that the gene duplications that gave rise to the GnRH-type and corazonin-type signaling systems occurred shortly before the divergence of protostomes and deuterostomes, not allowing time for significant diversification. Thus, at this point of divergence the two peptide types may have been very similar or even identical. If this hypothesis is correct, it may explain why there has been uncertainty in assigning names to GnRH/AKH/ACP/corazonin-type peptides in invertebrates, as discussed in more detail below. Furthermore, it is clear from our work on GnRH/corazonin-type receptors in starfish (Tian et al., 2016) that the only sure way to classify a GnRH/corazonin-type peptide is to identify the receptor type that it activates.

3. Discovery of distinct GnRH-type and CRZ-type signaling pathways in starfish provides a basis for revision and standardization of nomenclature for GnRH/corazonin-type peptides

3.1. Proposed standardized nomenclature for GnRH-type and corazonin-type neuropeptides

Our discovery of distinct GnRH-type and corazonin-type signaling pathways in a deuterostomian invertebrate, the starfish *A. rubens* (Tian et al., 2016), provides a basis for establishing a standardized nomenclature for GnRH-type and corazonin-type neuropeptides in protostomes and deuterostomes. We propose that neuropeptides in this family are classified as either GnRH-type or corazonin-type, with a definitive identification being based only on the receptor type that the peptide activates. It is clear that GnRH/corazonin-type receptors form two distinct clades – a GnRH-type receptor clade and corazonin-type receptor clade – as can be seen in Fig. 2 (Tian et al., 2016) and in other trees reported previously (Roch et al., 2014a; Roch et al.,

2014b; Zandawala et al., 2015b; Kavanaugh and Tsai, 2016). Therefore, the neuropeptide ligands for these two receptor types should be named accordingly. We propose that, contrary to other suggestions (Roch et al., 2014a; Roch et al., 2014b; Plachetzki et al., 2016), GnRH (not AKH or ACP) and corazonin (not corazonin/GnRH or InvGnRH) are the most appropriate names for peptides in non-arthropod species because these were the names assigned to the first of these two neuropeptide types to be discovered (Amoss et al., 1971; Schally et al., 1971; Veenstra, 1989).

Thus, the ligand for a GnRH-type receptor in a nematode or a mollusk or an annelid or an echinoderm or a cephalochordate (or indeed in any other bilaterian phylum, except arthropods – see below) should be referred to as GnRH. The name "GnRH" does not imply anything about function though, because GnRH-type peptides may not act as gonadotropin-releasing hormones in most, and possibly all, invertebrates due to the lack of hypothalamic–pituitary–gonadal axis and orthologs of vertebrate gonadotropins (Roch et al., 2012; Minakata and Tsutsui, 2016). Instead the name GnRH is simply used to indicate an orthologous relationship with the prototypical GnRH peptide that was first discovered in mammals. An exception to use of the name GnRH for neuropeptides of this type is in the arthropods. Here, duplication of an ancestral GnRH-type signaling system has given rise to the paralogous AKH/RCPH-type and ACP-type signaling systems (Hauser and Grimmelikhuijzen, 2014). Therefore, we propose that the names AKH, RPCH and ACP continue to be used for peptides of these types in the arthropods. However, when using the name ACP it should be recognized that neuropeptides of this type are evolutionarily more closely related to AKH than to corazonin. Furthermore, we recognize that the possibility remains that duplication of the GnRH signaling system may not be unique, amongst the invertebrates, to arthropods. Thus, duplication of the GnRH system may have also occurred in other invertebrates, in which case a suitable nomenclature would need to be devised. For example, there are four distinct genes encoding peptides belonging to the GnRH/corazonin superfamily in the annelid *Platynereis dumerelii*, the receptors for which await functional characterization (Conzelmann et al., 2013a). Conversely, in the nematode *Caenorhabditis elegans* there are at least two GnRH-type receptors, but only one of these

has been functionally characterized as a GnRH receptor with ligand identification (Vadakkadath Meethal et al., 2006; Lindemans et al., 2009). It is possible that these additional ligands and/or receptors represent distinct signaling systems that remain uncharacterized.

Likewise, the ligand for a corazonin-type receptor in a mollusk or an annelid or an echinoderm or a cephalochordate (or indeed in any other phylum) should be referred to as corazonin. As with GnRH, the name does not imply anything about function. Indeed, it is clear that even in insects corazonin does much more than excite the heart (the effect that provided the basis for the Spanish-inspired name) (Veenstra, 2009; Boerjan et al., 2010). However, "corazonin" is the prototype for a family of neuropeptides that occur in both protostomes and deuterostomes and therefore we propose that this name should be used for all members of this neuropeptide family. Accordingly, we propose that alternative names that have been used for members of this neuropeptide family, such as "invertebrate GnRH" (Roch et al., 2014a) or "corazonin/GnRH" (Hauser and Grimmelikhuijzen, 2014; Roch et al., 2014b; Semmens et al., 2016), should be discontinued.

To illustrate application of the proposed standardized nomenclature for GnRH/corazonin-type neuropeptides, below we highlight selected examples from the literature where a revision in the naming of neuropeptides and/or receptors is, in our opinion, necessary. Our highlighting of these examples from the literature should not be interpreted as a criticism of the authors of the selected papers. Making sense of the evolutionary relationships of GnRH/corazonin-type neuropeptides has proven to be very difficult and it is only with the availability of data from wider range of phyla that some key insights have been obtained recently – for example, the occurrence of corazonin-type signaling in deuterostomes. In addition to the text below, we present in Table 1 a list of GnRH/corazonin-type neuropeptides where a change in nomenclature is proposed. We hope this will be a useful resource for researchers working in this field of research.

3.2. Proposed nomenclature for ACP-type peptides in arthropods

As highlighted above, an ACP-type peptide was first discovered in the locust *L. migratoria* but it was named *L. migratoria* hypertrehalosaemic hormone (Lom-HrTH) based on sequence similarity with the *Drosophila* AKH/HrTH (Siegert, 1999). Related peptides were discovered in *Anopheles gambiae, Tribolium castaneum*, *Bombyx mori, Aedes aegypti* and *Culex pipiens* (Table 1). Based on receptor deorphanisation studies for some of these peptides, it is now evident that they all belong to the family of ACP-type neuropeptides. Hence, it is proposed that they should be referred to as ACP and not with the name that was first assigned to them (Table 1).

3.3. Proposed nomenclature for nematode GnRH-type neuropeptides

The first member of the GnRH neuropeptide family to be identified in the phylum Nematoda was discovered in *C. elegans* (Lindemans et al., 2009). This peptide was referred to as Ce-AKH-GnRH because it has structural features similar to arthropod AKH but acts as a ligand for a GnRH-type receptor. Furthermore, the peptide has functional similarity with mammalian GnRH because it regulates egg-laying behavior, a reproductive process that is comparable to the gonadotropic actions of GnRH. As highlighted above, the paralogous AKH-type and ACP-type signaling systems arose by duplication of a GnRH-type signaling system in the arthropod lineage (Hauser and Grimmelikhuijzen, 2014), and so it can be argued that use of the name AKH outside of the arthropods is inappropriate. We propose, therefore, that Ce-AKH-GnRH is renamed *C. elegans* GnRH or CeGnRH and likewise for orthologous peptides in other nematodes (Table 1).

3.4. Proposed nomenclature for GnRH-type and corazonin-type peptides in lophotrochozoans

The first lophotrochozoan GnRH/corazonin-type peptide to be identified was isolated from the mollusk *Octopus vulgaris* and a cDNA encoding the precursor of this peptide was also sequenced (Iwakoshi et al., 2002). Because it exhibited structural similarity with vertebrate GnRH-type peptides and also mimicked the effect of mammalian GnRH in an assay measuring lutenizing hormone release, the *Octopus* peptide was referred to as a GnRH-like peptide. Accordingly, when

447 the receptor for the *Octopus* peptide was identified it was referred to as a GnRH receptor (Kanda et
448 al., 2006). Likewise, when an ortholog of the *Octopus* receptor was functionally characterized more
449 recently in the sea hare *Aplysia californica*, it was also referred to as a GnRH receptor (Tsai et al.,
450 2010; Kavanaugh and Tsai, 2016). However, phylogenetic analysis has revealed that the *Octopus*
451 "GnRH receptor" and orthologs of this receptor in other mollusks are more closely related to
452 arthropod corazonin receptors than to vertebrate GnRH receptors or arthropod AKH/ACP-type
453 receptors (Fig. 2) (Roch et al., 2014b). This finding led to a partial revision of nomenclature, with
454 reference to the *Octopus* peptide and its orthologs as corazonin/GnRH or Crz/GnRH. Accordingly,
455 the receptors for these peptides were named CrzR/GnRHR (Hauser and Grimmelikhuijzen, 2014;
456 Roch et al., 2014b). We propose that nomenclature revision should progress one step further by
457 abandoning reference to GnRH. Thus, the peptides formerly known as *Octopus* GnRH or *Aplysia*
458 GnRH should be referred to as *Octopus* corazonin and *Aplysia* corazonin, with their cognate
459 receptors referred to as corazonin receptors (Table 1).
460 Subsequent to the discovery of *Aplysia* "GnRH" (i.e. *Aplysia* corazonin in the proposed new
461 nomenclature), a second GnRH/AKH-like peptide was identified in *Aplysia* (Johnson et al., 2014)
462 and named *Aplysia* adipokinetic hormone or *Aplysia* AKH. Receptors for an ortholog of this peptide
463 have been functionally characterised in the bivalve mollusk *Crassostrea gigas* and named *C. gigas*
464 AKH receptors (Dubos et al., 2016; Li et al., 2016). As highlighted above with respect to
465 nematodes, we propose that the name AKH should be restricted to insects/arthropods and therefore
466 molluscan "AKH" peptides and "AKH receptors" should instead be named GnRH and GnRH
467 receptors. In accordance with this proposal, a phylogenetic analysis reported by Li et al. (2016)
468 shows that the newly named *C. gigas* GnRH receptors belong to a clade of receptors that includes
469 closely related receptors from other lophotrochozoans, arthropod AKH/ACP-type receptors and
470 deuterostomian GnRH-type receptors (Li et al., 2016).
471
472 **4. Future directions for research on the evolution of GnRH/corazonin signaling**

In figure 3 we show the sequence of events that we propose gave rise to GnRH/AKH/ACP/CRZ signaling systems that occur in extant animals, with supporting evidence from receptor deorphanisation experiments. Thus, duplication of an ancestral GnRH/corazonin-type signaling system occurred in a common ancestor of protostomes and deuterostomes, which ultimately gave rise to the paralogous GnRH-type and corazonin-type signaling systems. Then a second duplication of the GnRH signaling system gave rise to the AKH-type and ACP-type signaling systems found in arthropods. If this hypothesis is correct, then what remains to be discovered? In figure 3 we incorporate information from a total of just seven phyla – Chordata, Hemichordata, Echinodermata, Annelida, Mollusca, Nematoda and Arthropoda. However, at least thirty-three extant phyla are recognized (Holland, 2011); the majority of these are protostomes and include ecdysozoan phyla such as Tardigrada and Priapulida and lophotrochozoan phyla such as Brachiopoda and Platyhelminthes. Based on analysis of genome/transcriptome sequence data, GnRH/corazonin-type peptides have recently been identified in tardigrade, priapulid and brachiopod species, but not in Platyhelminthes (Hauser and Grimmelikhuijzen, 2014; Li et al., 2016). Now it will be interesting to identify the receptors for these peptides and to investigate the physiological roles of GnRH/corazonin-type signaling in these phyla. Another phylum that is of particular interest is the phylum Xenoacoelomorpha, which includes acoels, nemertodermatids and the mysterious *Xenoturbella* (Cannon et al., 2016). The phylogenetic position of this phylum in the animal kingdom is controversial, with some authors proposing a deuterostomian affinity (Philippe et al., 2011) and others proposing that this bilaterian phylum is a sister group to the protostome plus deuterostome assemblage (nephrozoa) (Cannon et al., 2016). Either way, analysis of this phylum may provide interesting new insights into the evolution of GnRH/corazonin-type signaling.

Reconstructing the molecular evolution of neuropeptide signaling systems is important because it provides a framework for the more challenging and arguably even more interesting objective of reconstructing the evolution of neuropeptide function. With respect to

GnRH/corazonin-type signaling, our knowledge of neuropeptide function is currently skewed towards studies on vertebrates and insects. Nonetheless, now in the post-genomic era it is noticeable that insights into the physiological roles of GnRH/corazonin-type signaling in other animal types are emerging – for example, the physiological roles of a GnRH signaling system as a regulator of egg-laying behavior in the nematode *C. elegans* have been revealed (Lindemans et al., 2009). Furthermore, unlike the AKH signaling system in insects, the GnRH signaling in *C. elegans* does not appear to play any role in the regulation of lipid levels (Lindemans et al., 2009). It remains to be seen if carbohydrates and proline levels are affected by *C. elegans* GnRH as is the case with some insect AKHs (Yeoh et al., 2017) (http://www.neurostresspep.eu/diner/insectneuropeptides). Beyond insects, very little is known about the physiological roles of the corazonin-type signaling system, which in part reflects the loss of this signaling system in several major animal groups that include vertebrates, urochordates and nematodes. However, insights into corazonin-type neuropeptide function in non-insects can be found if the literature is scanned through the lens of the revised neuropeptide nomenclature proposed here (see above and Table 1). Thus, the neuropeptide designated originally as "GnRH" in the mollusk *Aplysia californica* is in fact the ligand for a corazonin-type receptor (Kavanaugh and Tsai, 2016) and hence we have proposed that it should be named *Aplysia californica* corazonin (or AcCRZ). The physiological roles of this peptide have been investigated in detail, revealing that it has no effect on ovotestis mass, reproductive tract mass, egg-laying, and penile eversion. It also has no effect on oocyte growth and egg-laying hormone accumulation and secretion. However, the peptide triggers parapodial opening, inhibition of feeding, and promotion of substrate attachment (Tsai et al., 2010). Consistent with these wide-ranging actions, analysis of the expression of the peptide revealed a widespread pattern of expression in the central nervous system, but most notably in the pedal, cerebral and abdominal ganglia (Jung et al., 2014). Similarly, the *Octopus vulgaris* corazonin (originally referred to as Oct-GnRH) is also widely distributed in the nervous system and regulates multiple functions including the stimulation of heart, oviduct and radula retractor muscle contractility (Iwakoshi et al., 2002;

525 Iwakoshi-Ukena et al., 2004; Kanda et al., 2006; Minakata and Tsutsui, 2016). Furthermore, its
526 receptor (originally referred to as Oct-GnRHR) is expressed in several peripheral tissues and
527 regions of the nervous system that are associated with autonomic functions, feeding, memory and
528 movement (Kanda et al., 2006). The physiological roles of corazonin-type peptides in mollusks can
529 be compared with what is known about corazonin function in insects, where roles in heart and
530 reproductive tissue contractility, and feeding have been discovered (Veenstra, 1989; Tayler et al.,
531 2012; Patel et al., 2014; Kubrak et al., 2016) .

532 The effects of a GnRH-type peptide (referred to as *Aplysia* AKH) have also been
533 investigated in *Aplysia californica*. Transcripts encoding the peptide were found to be expressed in
534 abdominal, cerebral, and pleural ganglia, but peptide-containing processes were observed in all
535 ganglia, indicating a widespread role as a neuromodulator. Accordingly, injection of the peptide
536 inhibited feeding, reduced body mass, increased excretion of feaces, and reduced gonadal mass and
537 oocyte diameter (Johnson et al., 2014). Comparison of the actions of GnRH-type and corazonin-
538 type neuropeptides in mollusks indicates that they have overlapping functions as well as some
539 functions specific to each signaling system (see above and (Tsai et al., 2010)). When a similar
540 comparison is performed in arthropods, it appears that the degree of functional overlap between the
541 two signaling systems varies from one lineage to another. Hence in the crayfish *Procambarus*
542 *clarkii*, both RPCH and corazonin regulate pigment migration (Porras et al., 2001; Porras et al.,
543 2003) and in *Drosophila* both AKH and corazonin influence metabolic stresses (Bharucha et al.,
544 2008; Zhao et al., 2010; Galikova et al., 2015; Kubrak et al., 2016). Furthermore, in the stick insect
545 *Baculum extradentatum* AKH regulates heart contractility (Malik et al., 2012). However, in
546 *Rhodnius prolixus*, only corazonin has cardioacceleratory effects and only AKH regulates lipid levels
547 (Patel et al., 2014). And so we can see here from studies on mollusks and arthropods how the
548 paralogous GnRH-type and corazonin-type signaling systems may have retained some ancestral
549 functions (such as the modulation of stress and metabolism) from a common ancestral molecule,
550 whilst also acquiring some distinct physiological roles. It remains to be determined, however,

whether or not additional comparisons of neuropeptide function in different phyla will reveal evidence of physiological roles that are specific for GnRH-type peptides on the one hand and corazonin-type peptides on the other. To address this issue, what is needed now are more experimental studies that compare the functions of GnRH-type and corazonin-type signaling in other animal phyla where both of these systems have been retained. With our recent discovery of distinct GnRH-type and corazonin-type signaling systems in an echinoderm, the starfish *A. rubens* (Tian et al., 2016), there is an opportunity to do this.

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Peptide sequence	Species	Original name	Revised name	Receptor deorphan.	Reference
Arthropoda ACP					
pQVTFSRDWSPa	*Locusta migratoria*	Lom-HrTH	ACP	No	(Siegert, 1999)
pQVTFSRDWNAa	*Anopheles gambiae*	AKH-II	ACP	Yes	(Kaufmann and Brown, 2006; Hansen et al., 2010)
pQVTFSRDWNPa	*Tribolium castaneum*	AKH-3	ACP	Yes	(Li et al., 2008; Hansen et al., 2010)
pQITFSRDWSGa	*Bombyx mori*	AKH-3	ACP	Yes	(Roller et al., 2008; Shi et al., 2011)
pQVTFSRDWNAa	*Aedes aegypti*	AKH-II	ACP	No	(Kaufmann et al., 2009)
pQVTFSRDWNAa	*Culex pipiens*	AKH-II	ACP	No	(Kaufmann et al., 2009)
Nematoda GnRH					
pQMTFTDQWT	*Caenorhabditis elegans*	Ce-AKH-GnRH	GnRH	Yes	(Lindemans et al., 2009)
pQMTFSDGWa	*Globodera rostochiensis*	AKH	GnRH	No	(Li et al., 2016)
Lophotrochozoa GnRH					
pQVSFS-TN-WGSa	*Crassostrea gigas*	AKH	GnRH	Yes	(Hauser and Grimmelikhuijzen, 2014; Li et al., 2016)
pQIHFS-PD-WGTa	*Aplysia californica*	AKH	GnRH	No	(Hauser and Grimmelikhuijzen, 2014)
pQIHFS-PT-WGSa	*Lottia gigantea*	AKH	GnRH	No	(Hauser and Grimmelikhuijzen, 2014; Roch et al., 2014b)
pQISFS-TN-WGSa	*Hyriopsis cumingii*	AKH	GnRH	No	(Hauser and Grimmelikhuijzen, 2014)
pQIHFT-PG-WGSa	*Bithynia siamensis goniomphalos*	AKH	GnRH	No	(Hauser and Grimmelikhuijzen, 2014)
pQIHFS-PG-WEPa	*Tritonia diomedea*	AKH	GnRH	No	(Hauser and Grimmelikhuijzen, 2014)
pQISFS-TD-WGSa	*Mytilus galloprovincialis*	AKH	GnRH	No	(Li et al., 2016)
pQFSFSLPGKWGNa	*Platynereis dumerilii*	AKH-1	GnRH-1	No	(Conzelmann et al., 2013a)
Lophotrochozoa CRZ					
pQNYHFSNGWHPGa	*Octopus vulgaris*	GnRH	CRZ	Yes	(Iwakoshi et al., 2002)
pQNYHFSNGWYA-a	*Aplysia californica*	GnRH	CRZ	Yes	(Zhang et al., 2008; Kavanaugh and Tsai, 2016)
pQNYHFSNGWQP-a	*Crassostrea gigas*	GnRH	CRZ	No	(Bigot et al., 2012; Stewart et al., 2014)
pQHYHFSNGWKS-a	*Lottia gigantea*	GnRH	CRZ	No	(Veenstra, 2010)
pQAYHFSHGWFP-a	*Capitella teleta*	GnRH-1	CRZ	No	(Veenstra, 2011)
pQSYHFSNGWNP-a	*Ruditapes philippinarum*	GnRH	CRZ	No	(Song et al., 2015)
pQNFHYSNGWQP-a	*Patinopecten yessoensis*	GnRH	CRZ	No	(Nagasawa et al., 2015)
pQAYHFSNGWMP-a	*Platynereis dumerilii*	GnRH-1	CRZ	No	(Conzelmann et al., 2013a)
Cephalochordata CRZ					
pQILCARAFTYTHTWa or FTYTHTWa	*Branchiostoma floridae*	GnRH	CRZ	Yes (partially)	(Roch et al., 2014b; Tian et al., 2016)

Table 1. Table showing the sequences of GnRH/AKH/ACP/CRZ-type peptides (column 1), with conserved residues highlighted in yellow, from a variety of invertebrate species (column 2). Proposed name changes are shown in columns 3 and 4, in accordance with the revised nomenclature presented in this review. Column 5 indicates whether or not the receptor for each peptide has been identified based on use of receptor deorphanisation assays. Column 6 shows the corresponding citations.

573
574 **FIGURE LEGENDS**

575

576 **Figure 1. Timeline highlighting some of the key discoveries in the history of research on the**
577 **evolution of GnRH-type and corazonin-type signaling.** Discoveries relating to GnRH signaling
578 are represented in red, discoveries relating to the paralogous GnRH-type signaling systems in
579 arthropods are shown in orange (AKH) or pink (ACP) and discoveries relating to corazonin (CRZ)
580 signaling are shown in purple. The relevant citations are as follows: GnRH discovered in mammals
581 (Baba et al., 1971; Schally et al., 1971); RPCH discovered in a crustacean (Fernlund, 1974); AKH
582 discovered in an insect (Stone et al., 1976); human GnRH precursor sequenced (Seeburg and
583 Adelman, 1984); locust AKH precursor sequenced (Schulz-Aellen et al., 1989); mouse GnRH
584 receptor sequenced (Tsutsumi et al., 1992); GnRH-type peptides discovered in a urochordate
585 (Powell et al., 1996); *Drosophila* GnRH-type receptor sequenced (Hauser et al., 1998); locust ACP
586 discovered as LomHrTH (Siegert, 1999); AKH identified as the ligand for *Drosophila* GnRH-type
587 receptor (Staubli et al., 2002); mosquito ACP precursor sequenced (Kaufmann and Brown, 2006);
588 ligand for GnRH-type receptor identified in *C. elegans* (Lindemans et al., 2009); ACP receptors
589 discovered in insects (Hansen et al., 2010); ligands for GnRH-type receptors identified in a mollusk
590 and an echinoderm (Li et al., 2016; Tian et al., 2016) CRZ discovered in American cockroach
591 (Veenstra, 1989); *Drosophila* CRZ precursor sequenced (Veenstra, 1994); *Octopus* CRZ-type
592 peptide and precursor sequenced (Iwakoshi et al., 2002); *Drosophila* CRZ receptor sequenced
593 (Cazzamali et al., 2002; Park et al., 2002); ligand for *Octopus* CRZ-type receptor identified (Kanda
594 et al., 2006); partial characterization of ligand for an amphioxus CRZ-type receptor (Roch et al.,
595 2014b); ligand for CRZ-type receptor identified in an echinoderm (Tian et al., 2016).

596

597 **Figure 2. Phylogenetic analysis of GnRH/AKH/ACP/CRZ-type receptors reveals two distinct**
598 **clades – a GnRH/AKH/ACP-type receptor clade and a CRZ-type receptor clade.** GnRH-type
599 receptors are labelled using red squares, AKH-type receptors using orange squares, ACP-type

receptors using pink squares and CRZ-type receptors using purple circles. Neuropeptide S and CCAP receptors were used as an outgroup (condensed). The stars represent posterior probabilities and the pastel coloured backgrounds represent different groups of animals (see key). The scale bar indicates amino acid substitutions per site. Species for which receptor-ligand interactions have been experimentally characterized are coloured in green, including the *A. rubens* receptors characterized in this study (boxed in black). Species names are as follows: A.rub, *Asterias rubens*; S.pur, *Strongylocentrotus purpuratus*; B.flo, *Branchiostoma floridae*; H.sap, *Homo sapiens;* D.rer, *Danio rerio*; G.gal, *Gallus gallus*; C.tel, *Capitella teleta*, C.gig, *Crassostrea gigas*; L.gig, *Lottia gigantea*; S.mar, *Strigamia maritima*; D.pul, *Daphnia pulex*; B.mor, *Bombyx mori*; R.pro, *Rhodnius prolixus*; A.gam, *Anopheles gambiae*; I.sca, *Ixodes scapularis*; S.kow, *Saccoglossus kowalevskii*; O.vul, *Octopus vulgaris*. [This figure is reproduced from (Tian et al., 2016)].

Figure 3. Diagram showing the evolution of GnRH-type and CRZ-type receptors. GnRH-type receptors (red) and CRZ-type receptors (purple) arose by gene duplication in a common ancestor of the protostomes and deuterostomes. A second gene duplication of a GnRH-type receptor in a common ancestor of the Arthropoda gave rise to AKH-type receptors (orange) and ACP-type receptors (pink). CRZ-type receptors have been lost in multiple lineages (purple crosses), including vertebrates, and the ACP-type receptor has been lost in *Drosophila* and the crustacean *Daphnia* (pink cross). The occurrence of each receptor type in different species is shown on the right, with a white box denoting loss of a receptor. A black question mark indicates that a receptor type has not been found but because complete genome sequence data are not available it is not possible at present to conclude whether or not the receptor has been lost. Species where neuropeptide ligands for receptors have been identified are labeled with a yellow asterisk. The ? in the CRZR box for *Branchiostoma floridae* indicates uncertainty regarding the structure of a candidate ligand, as discussed in this review and (Tian et al., 2016).

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GnRH, AKH, ACP and CRZ discovery timeline


GnRH discovered in mammals — 1971


RPCH discovered in a crustacean — 1972


AKH discovered in an insect — 1976


Human GnRH precursor sequenced — 1984


Locust AKH precursor sequenced — 1989

CRZ discovered in American cockroach


Mouse GnRH receptor sequenced — 1992

1994 — *Drosophila* CRZ precursor sequenced


GnRH-type peptides discovered in a urochordate — 1996


Drosophila GnRH-type receptor sequenced — 1998


Locust ACP discovered as *LomHrTH* — 1999


AKH identified as the ligand for *Drosophila* GnRH-type receptor — 2002

Octopus CRZ-type peptide and precursor sequenced

Drosophila CRZ receptor discovered


Mosquito ACP precursor sequenced — 2006

Ligand for *Octopus* CRZ-type receptor identified


Ligand for GnRH-type receptor identified in *C. elegans* — 2009

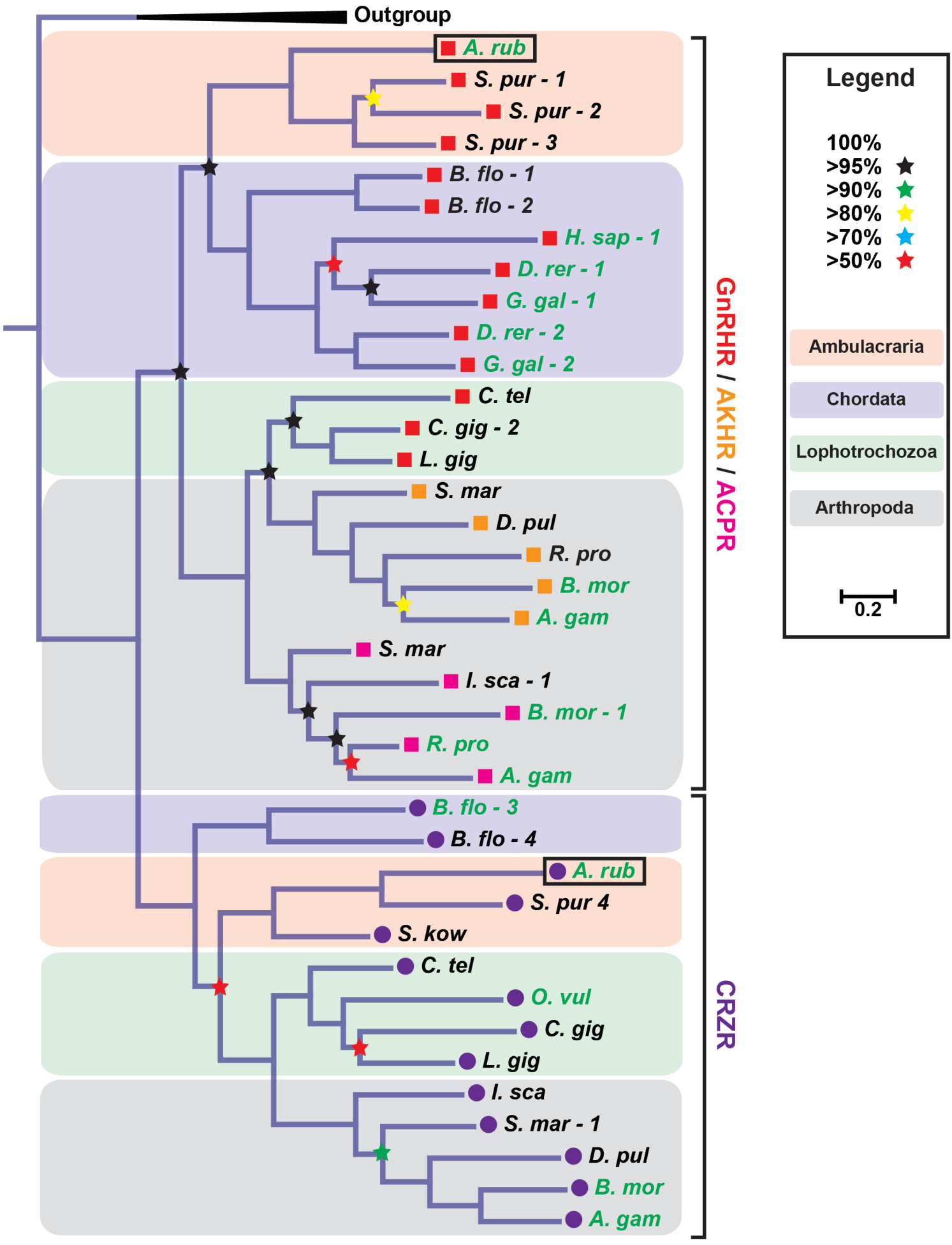

ACP receptors discovered in insects — 2010

2014 — Partial characterization of ligand for an Amphioxus CRZ-type receptor


Ligands for GnRH-type receptors identified in a mollusk and in an echinoderm — 2016

Ligand for CRZ-type receptor identified in an echinoderm

Present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S. pur - 3
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G. gal - 2
C. tel
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L. gig
S. mar
D. pul
R. pro
B. mor
A. gam
S. mar
I. sca - 1
B. mor - 1
R. pro
A. gam
B. flo - 3
B. flo - 4
A. rub
S. pur 4
S. kow
C. tel
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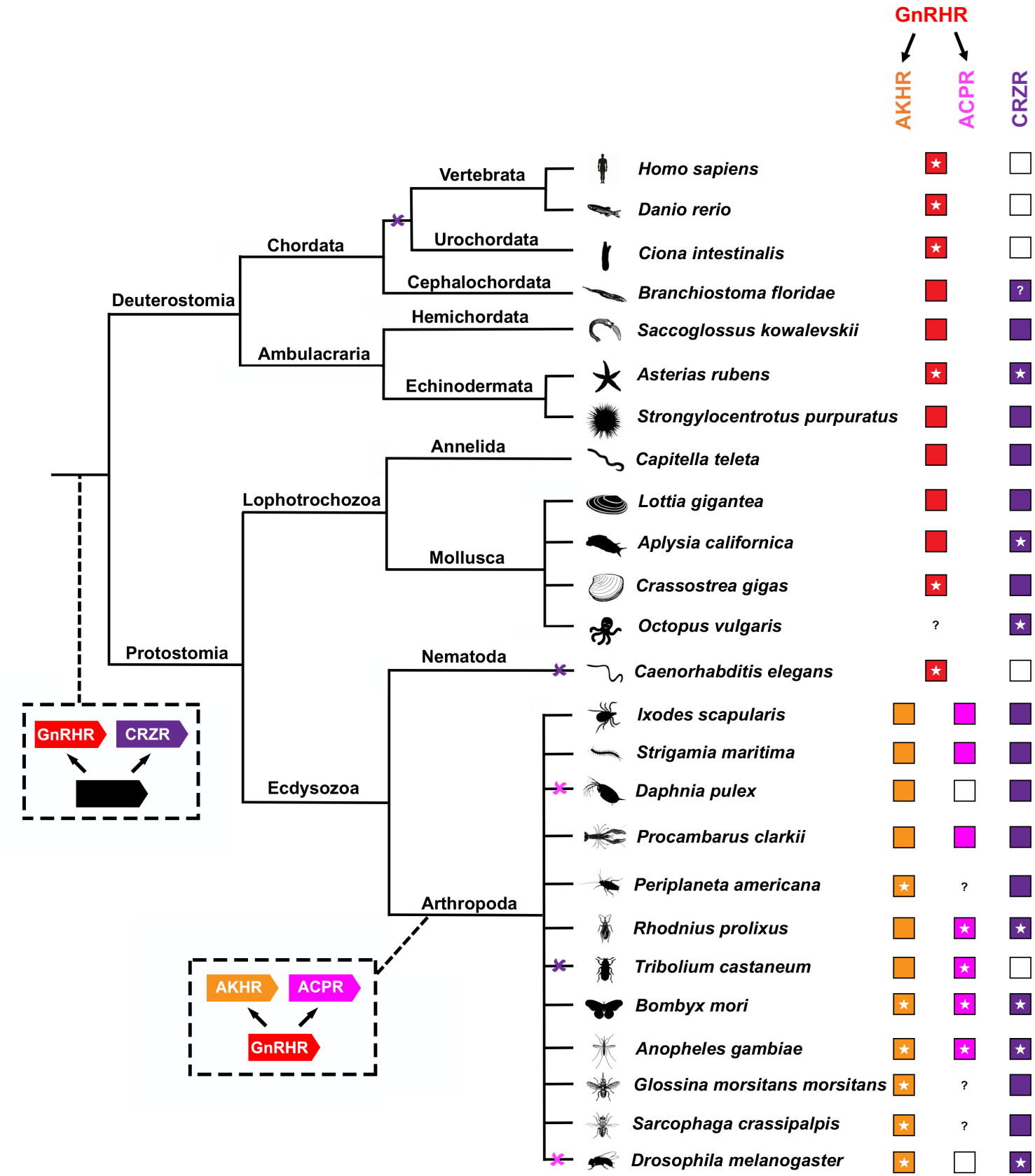
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Chordata
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Cephalochordata
Ambulacraria
Hemichordata
Echinodermata
Protostomia
Lophotrochozoa
Annelida
Mollusca
Ecdysozoa
Nematoda
Arthropoda
GnRHR
CRZR
AKHR
ACPR
GnRHR
Homo sapiens
Danio rerio
Ciona intestinalis
Branchiostoma floridae
Saccoglossus kowalevskii
Asterias rubens
Strongylocentrotus purpuratus
Capitella teleta
Lottia gigantea
Aplysia californica
Crassostrea gigas
Octopus vulgaris
Caenorhabditis elegans
Ixodes scapularis
Strigamia maritima
Daphnia pulex
Procambarus clarkii
Periplaneta americana
Rhodnius prolixus
Tribolium castaneum
Bombyx mori
Anopheles gambiae
Glossina morsitans morsitans
Sarcophaga crassipalpis
Drosophila melanogaster