



Mosquito Peptide Hormones: Diversity, Production, and Function

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Abstract

Mosquitoes, like other insects, produce a diversity of peptide hormones that are processed from different precursor proteins and have a range of activities. Early studies relied on purification of bioactive peptides for hormone identification, but more recently genomic data have provided the information needed to more comprehensively identify peptide hormone genes and associated receptors. The first part of this chapter summarizes the known or predicted peptide hormones that are produced by mosquitoes. The second part of this chapter discusses the sources of these molecules and their functions.



1. INTRODUCTION

All multicellular organisms produce peptide hormones that are classified on the basis of shared structural features and/or function (Fricker, 2012). Most derive from precursors called propeptides that are produced and

enzymatically processed in different types of endocrine or neurosecretory cells. Endocrine cells usually store and directly release peptide hormones into circulation, whereas neurosecretory cells can either release hormones directly or transport them through axons to neurohemal organs or other locations where they are stored and released (Nässel and Winther, 2010; Szapiro and Barbour, 2009). Peptide hormones mediate activities by binding to membrane receptors that activate intracellular signalling and effector functions in different types of cells. A majority of peptide hormones bind receptors in the large G protein-coupled receptor (GPCR) family, but some bind protein kinase receptors (PKRs) or receptor guanylyl cyclases (RGCs) (Biarç et al., 2011; Lemmon and Schlessinger, 2010; Potter, 2011). The focus of this chapter is the peptide hormones of mosquitoes. We first discuss the known or predicted peptide hormones mosquitoes produce and the receptors they bind. We then examine the sources of these hormones and their functions. Insects, including mosquitoes, use some peptide hormones as neurotransmitters, while also producing other peptides that have important roles in development, locomotion, circadian rhythms, olfaction, and mating. We largely do not discuss these topics because of space constraints and/or the limited mosquito literature that is available.



2. PEPTIDE HORMONES AND RECEPTOR DISCOVERY IN MOSQUITOES

The first endocrine studies in mosquitoes began in the mid-20th century with experiments showing that a humoral factor released from the head of female mosquitoes stimulated development of eggs after blood feeding (Clements, 1956; Detinova, 1945; Gillett, 1956). This factor was initially named egg development neurosecretory hormone (EDNH) because experiments showed it was produced by brain medial secretory cells (Lea, 1967, 1972). Studies later established that EDNH was a protein or small peptide that stimulated ovaries of *Aedes aegypti* to produce the steroid hormone ecdysone, which was shown to be essential for yolk biosynthesis by the fat body, yolk uptake by primary oocytes and egg maturation (Hagedorn et al., 1975, 1979; Lea, 1972) (see chapter “Regulation of Reproductive Processes in Female Mosquitoes” by Roy et al.). This finding resulted in the renaming of EDNH as ovary ecdysteroidogenic hormone (OEH) (Matsumoto et al., 1989a). However, identification of OEH as a 140 amino acid peptide required many years of effort because large quantities of starting material from mosquito heads were needed to ultimately purify the bioactive peptide and determine its sequence (Brown et al., 1998).

Other studies during this period provided evidence that mosquitoes produce peptide hormones with functions in nutrient storage (Lea and Van Handel, 1970), water balance and diuresis (Maddrell and Phillips, 1978; Nijhout and Carrow, 1978), host seeking (Klowden and Lea, 1979), and blood meal digestion (Graf et al., 1998). Yet the small size of mosquitoes presented similar difficulties to those confronted with OEH. As a result, few of these factors were identified. Instead, most peptide hormones that were successfully purified and identified came from larger insects like certain moths, locusts, and cockroaches (Gäde et al., 1997; Hauser et al., 1997, 2006a,b; Raabe, 1989). In some cases, these sequence data were then used to identify homologous peptide sequences from mosquitoes (Veenstra, 1994, 1999; Veenstra et al., 1997a,b, 1999).

Approaches to studying peptide hormones and their receptors dramatically changed with the sequencing and annotation of the first insect genome from *Drosophila melanogaster* (Adams et al., 2000). This was soon followed by the sequencing of the mosquito, *Anopheles gambiae* (Holt et al., 2002), and several other mosquito species including *Ae. aegypti* and *Culex quinquefasciatus* (Arensburger et al., 2010; Jiang et al., 2014; Neafsey et al., 2015; Nene et al., 2007; Waterhouse, 2015). These data together with the genomes for several other insects, invertebrates, and vertebrates provided the foundation needed to identify candidate peptide hormone and receptor genes through homology-based searches (Hauser et al., 2010; Hewes and Taghert, 2001; Hummon et al., 2006; Li et al., 2008; Predel et al., 2010; Riehle et al., 2002; Roller et al., 2008; Southey et al., 2008; Veenstra et al., 2012).

2.1 Peptide Hormone Genes

Annotation of the *Ae. aegypti* and *An. gambiae* genomes originally identified 43 and 35 peptide hormone genes, respectively (Predel et al., 2010; Riehle et al., 2002). Additions to the literature since these studies were published together with annotation improvements increase this number to 50 for *Ae. aegypti*, 47 for *An. gambiae*, and 45 *C. quinquefasciatus*. We list these in alphabetical order in Table 1 along with their identifiers, which will be useful to readers because some of these genes are still difficult to find or correctly recognize in databases. We also list the 45 genes that have been identified from *D. melanogaster*, which is of value to this discussion from two perspectives. First, more peptide hormones have been functionally studied in *D. melanogaster* than in any mosquito species. Second, mosquitoes and *D. melanogaster* reside in families (Culicidae and Drosophilidae) that arose

at different times in the evolutionary history of the order Diptera. The Culicidae is an early lineage that arose 225 million years ago (mya) while the Drosophilidae evolved much more recently (40–65 mya) in concert with other cyclorrhaphan flies (Wiegmann et al., 2003). Thus, the peptide hormone genes from mosquitoes and drosophilids together provide information across the breadth of the order.

2.1.1 Comparative Genomics

Most peptide hormone genes are single copy and produce mature hormones that are monomeric. However, differential processing can yield multiple bioactive forms for some peptide hormones with the most extreme example in mosquitoes being prepro-FMRamide, which is processed into nine functional FMRamide isoforms (Predel et al., 2010). Some peptide hormone genes along with their receptors are duplicated in mosquitoes and select other insects. These include adipokinetic hormone (AKH), corazonin, and AKH/corazonin peptide (ACP) (Table 1). Some of these duplications, however, have been lost in drosophilids including *D. melanogaster* (Hauser and Grimmelikhuijzen, 2014; Vogel et al., 2013). The only multimember peptide hormone genes in mosquitoes, drosophilids, and other insects are the insulin-like peptides (ILPs), but it is unclear all family members derive from a common ancestral gene (Grönke et al., 2010). Lastly, bursicon and glycoprotein A2/B5 are dimeric peptide hormones that are produced from cleavage products of different prepropeptides (bursicon/partner of bursicon and glycoprotein hormone A2/A5), whose corresponding genes are also expressed in different neurosecretory cells (Table 1). Trypsin modulating oostatic factor (TMOF) is a proline rich, 10 amino acid peptide identified in *Ae. aegypti* that is often discussed as a peptidyl regulator of blood meal digestion and egg formation in mosquitoes (Borovsky et al., 1990; Verlinden et al., 2014). However, TMOF is not included in Table 1 or further discussed here because it matches a 10 amino acid sequence of the vitelline envelope protein (Lin et al., 1993), which clearly is not a peptide hormone precursor protein. In addition, no data compellingly supports that TMOF binds a specific receptor including any GPCR, PRK, or RGC, or activates signalling in any target cell that is consistent with it functioning as a peptide hormone.

Overall, the data in Table 1 show *Ae. aegypti*, *An. gambiae*, and *C. quinquefasciatus* encode the same peptide hormone genes with two exceptions. First the short neuropeptide (sNPF) gene in *Ae. aegypti* has duplicated, which we refer to here as sNPF1 and sNPF2 (see Section 4.1.16),

Table 1 Peptide Hormone Genes for Three Mosquito Species and *D. melanogaster*^a

Hormone	<i>Ae. aegypti</i>	<i>An. gambiae</i>	<i>C. quinquefasciatus</i>	<i>D. melanogaster</i>	Mosquito Species with Reported Localization Data ^b
Adipokinetic hormone (AKH)	AAEL011996	AGAP008834	CPIJ000869	CG1171	Aa, Ag
Adipokinetic/corazonin peptide (ACP)	AAEL010950	AGAP002430	CPIJ001379	ND	Aa, Ag
Agatoxin-like neuropeptide	PA	AGAP007821	CPIJ000956	ND	
Allatostatin A (ASTA)	AAEL015251	AGAP003712	CPIJ008017	CG13633	Aa
Allatostatin C (ASTC)	AAEL005747	AGAP010157	partial unan.	CG14919	Aa
Allatotropin	AAEL009541	AGAP012130	CPIJ007896	ND	Aa
Apis-ITG-like	AAEL006369	AGAP008993	CPIJ005482	CG8216	
Bursicon	PA	AGAP002537	CPIJ009600	CG13419	Ag
Partner of bursicon	AAEL013722	AGAP004506	CPIJ012985	CG15284	Ag
CCHamide 1	AAGE02019353	BM585352	AAWU01008744	CG14358	
CCHamide 2	AAEL004890	AGAP004553	AAWU01038417	CG14375	
Corazonin	AAEL005252	AGAP012665	ND	CG3302	Aa
Crustacean cardioactive peptide (CCAP)	AAEL000630	AGAP009729	CPIJ005842	CG4910	Ag

Continued

Table 1 Peptide Hormone Genes for Three Mosquito Species and *D. melanogaster*—cont'd

Hormone	<i>Ae. aegypti</i>	<i>An. gambiae</i>	<i>C. quinquefasciatus</i>	<i>D. melanogaster</i>	Mosquito Species with Reported Localization Data
Diuretic hormone 31 (DH31)	AAEL008070	AGAP001382	ND	CG13094	Aa
Diuretic hormone 44 (DH44)	AAEL008292	AGAP003269	CPIJ008822	CG8348	Aa
Ecdysis triggering hormone (ETH)	AAEL001762	AGAP007062	CPIJ004945	CG18105	Aa
Ecdysis hormone	AAEL011229	AGAP010437	CPIJ011911	CG5400	
FMRFamide	AAEL013645	AGAP005518	CPIJ000101	CG2346	Aa
Glycoprotein A2	BN001241	AGAP008301	CPIJ013236	CG17878	
Glycoprotein B5	AAEL001474	AGAP008302	CPIJ013235	CG40041	
Insulin-like peptide (ILP) 1	AAEL000937	AGAP010605	CPIJ018051	CG14173	
ILP2	AAEL000960	AY324308	CPIJ018049	CG8167	
ILP3	DQ845751	AY324309	CPIJ018050	CG14167	Aa, Ag, As
ILP4	AAEL000932	AY324310	ND	CG6736	
ILP5	AAEL003000	AGAP003927	CPIJ001698	CG33273	
ILP6	DQ845755	AY324313	CPIJ003329	CG14049	
ILP7	DQ845757	AY324314	ND	CG13317	
ILP8	DQ845754	ND	ND	CG14059	

Ion transport peptide	AAEL015332	AGAP005055	CPIJ003972	CG13586	
Limostatin 1	AAEL008355	AGAP013197	ND	CG8317	
Limostatin 2	AAEL008359	ND	ND	ND	
Kinin (leucokinin)	AAEL010172	AGAP013518	CPIJ010343	CG13480	Aa
Myoinhibitory peptide	AAEL012139	AGAP000833	CPIJ802231	CG6456	Aa
Myosuppressin	AAEL007294	AGAP001474	CPIJ012769	CG6440	
Natalisin	AAEL003260	AGAP005277	CPIJ001072	CG34388	
Neuropeptide F	AAEL002733	AGAP004642	PA	CG10342	Aa
Neuropeptide-like peptides (NPLPs)	AAEL014708	AGAP010366	CPIJ014175	CG3441	Aa
Orcokinin	AAEL010172	AGAP012220	CPIJ010343	CG13565	
Ovary ecdysteroidogenic hormone (OEH)	AAEL004155	AGAP000108	CPIJ010626	ND	Aa, Ag
Pigment dispersing hormone	AAEL001754	AGAP005776	CPIJ004895	CG6496	
Proctolin	ND	ND	ND	CG7105	
Prothoracicotropic hormone (PTTH)	PA	AGAP000859	CPIJ003196	CG13687	
Pyrokinin 1 (PK1)	AAEL012060	AGAP002292	CPIJ005970	CG6371	Aa, As, Cp

Continued

Table 1 Peptide Hormone Genes for Three Mosquito Species and *D. melanogaster*—cont'd

Hormone	<i>Ae. aegypti</i>	<i>An. gambiae</i>	<i>C. quinquefasciatus</i>	<i>D. melanogaster</i>	Mosquito Species with Reported Localization Data
Pyrokinin 2 (PK2)	AAEL005444	AGAP000347	partial unan.	CG15520	Aa, As, Cp
RYamide	AAEL011702	AGAP006765	CPIJ008988	CG40733	
Short neuropeptide F 1 (sNPF1)	AAEL012542	DQ437578	CPIJ009049	CG13968	Aa
Short neuropeptide F 2 (sNPF2)	AF155738.1	ND	ND	ND	Aa
SIFamide	AAEL009858	AGAP007056	CPIJ004953	CG33527	Aa
Sulfakinin	PA	AGAP009275	CPIJ004208	CG18090	Aa
Tachykinin	AAEL006644	AGAP010014	Partial unan.	CG14734	Aa, Cs
Trissin	AAEL008756	AGAP012496	CPIJ016124	CG14871	

³Gene entries for each species are by their VectorBase, GenBank, or FlyBase identifier. *PA*: identification of a partial exon in the genome that is unannotated. *ND*: gene not detected in databases.

^bAa, *Aedes aegypti*; Ag, *Anopheles gambiae*; As, *An. stephensi*; Cp, *Culex pipiens*; and Cs, *C. salinarius*.

whereas *An. gambiae* and *C. quinquefasciatus* have only one sNPF gene. Second, *Ae. aegypti* has eight ILP genes while *An. gambiae* has seven and *C. quinquefasciatus* has five. Table 1 notes a few other instances of genes not being detected in *C. quinquefasciatus* but this is likely due to annotation issues. *D. melanogaster* lacks genes for ACP, allatotropin, and OEH but orthologs of all other peptide hormone genes in mosquitoes are present. Orthologs of most peptide hormone genes in mosquitoes and drosophilids are also present in insects in other orders. The proctolin gene is present in *D. melanogaster* and other insects but is absent in mosquitoes. A few other peptide hormone genes present in one or more insects from other orders are absent from both mosquitoes and drosophilids (Table 1). The latter could indicate these peptide hormones are absent from all of the Diptera.

2.1.2 Transcription and Peptidomics

Transcriptional profiling of individual genes has been reported for several peptide hormones in mosquitoes, but only a few studies report expression data in specific tissues or life stages. In *An. gambiae*, tissue and/or life stage expression data are available for AKH and ACP (Kaufmann and Brown, 2006, 2008), bursicon (Robertson et al., 2007), ILPs, (Arsic and Guerin, 2008; Krieger et al., 2004), neuropeptide F (NPF) (Garczynski et al., 2005), and sNPF (Garczynski et al., 2007). In *Ae. aegypti*, such data are available for AKH and ACP (Kaufmann et al., 2009), allatostatin A and C (ASTA and C) (Hernández-Martínez et al., 2005; Li et al., 2006; Veenstra et al., 1997a), allatotropin (Hernández-Martínez et al., 2005; Veenstra and Costes, 1999), ILPs (Brown et al., 2008; Riehle et al., 2006), kinin (Veenstra, 1994), NPF (Stanek et al., 2002), OEH (Brown et al., 1998), and sNPF2 (Stracker et al., 2002). Data are also available for what we name in this paper pyrokinin 2 (Choi et al., 2013) in *Ae. aegypti* (see Section 4.1.15) and prothoracicotropic hormone (PTTH) in *Culex pipiens* (Zhang and Denlinger, 2011). Profiling of multiple *An. gambiae* or *Ae. aegypti* genes through array based or RNAseq studies provide additional data on peptide hormone gene expression (Akbari et al., 2013; Baker et al., 2011; Matthews et al., 2016). The only expression data available for other mosquito species are for ILP genes in *C. pipiens* (Sim and Denlinger, 2009) and *Anopheles stephensi* (Marquez et al., 2011; Pietri et al., 2015), and tachykinins in *Culex salinarius* (Meola et al., 1998). Insights about prepropeptide processing are currently only available for *Ae. aegypti* through peptidomic analysis of the central nervous system and select other tissues (Predel et al., 2010; Siju et al., 2013). It is also important to note that the

accuracy of the above data depends on the quality of the reference genomes that were available at the time the above studies were conducted.

2.2 Peptide Hormone Receptors

Insights into the known or predicted receptors for mosquito peptide hormones derive from two lines of investigation. The first is experimental data in one or more mosquito species showing that a particular receptor is activated or required for the biological activity of a given peptide hormone. The second is homology-based studies of mosquito receptor genes relative to *D. melanogaster*, other insects, or other animals where experimental data on orthologs are available. While several studies discuss GPCRs that bind peptide hormones (see later), [Vogel et al. \(2013\)](#) provides the only comprehensive summary of the receptors in mosquitoes and drosophilids that are known or predicted to bind peptide hormones. Key features of these receptors are summarized below.

2.2.1 GPCRs

GPCRs form a very large protein family that is present in all multicellular animals. However, the first studies showing that peptide hormones are often ligands for GPCRs derive from experiments first conducted in mammals. GPCRs range from 40 to 60 kDa in size and reside as monomers in the plasma membrane of cells. All exhibit a standard topology which consists of three regions: a variable extracellular N-terminal domain, a conserved transmembrane domain with seven hydrophobic α -helices that functions as a ligand binding pocket, and an intracellular C-terminal domain that mediates signalling through interactions with G proteins ([Bockhaert and Pin, 1999](#)). GPCRs are further subdivided into six classes (A–F) of which only Class A (rhodopsin-like) and Class B (secretin-like) contain members that bind peptide hormones ([Attwood and Findlay, 1994](#); [Hauser et al., 2006a,b](#)).

The conserved transmembrane domain shared by GPCRs was used during annotation to interrogate the *D. melanogaster* genome. This led to identification of more than 200 predicted GPCR genes of which 44 were Class A and B members ([Brody and Cravchik, 2000](#); [Hewes and Taghert, 2001](#)). A few functional studies of GPCRs preceded sequencing of the *D. melanogaster* genome but most of these predicted GPCRs were ‘orphans’ that were subsequently targeted for study by many groups using different experimental approaches. This resulted in ‘deorphanization’ and identification of GPCRs that bind several of the peptide hormones listed in [Table 1](#)

(see Johnson et al., 2003; Nässel and Winther, 2010). The same approach was used to annotate the GPCR genes of *An. gambiae*, *Ae. aegypti*, and *C. quinquefasciatus* (Arensburger et al., 2010; Hill et al., 2002; Nene et al., 2007). However, experimental studies identifying mosquito GPCRs that bind peptide hormones are currently restricted in *Ae. aegypti* to ASTC (Mayoral et al., 2010), allatotropin (Hayes et al., 1997; Nouzova et al., 2012), kinins (Kersch and Pietrantonio, 2011; Kwon et al., 2012; Pietrantonio et al., 2005; Taneja-Bageshwar et al., 2009), NPF (Liesch et al., 2013), pyrokinin 2 (Choi et al., 2013), and sNPFs (Liesch et al., 2013). In anopheline mosquitoes, GPCRs that bind AKH, crustacean cardioactive peptide (CCAP), and corazonin (Belmont et al., 2006), ACP (Hansen et al., 2010), myosuppressin (Scholler et al., 2005), NPF (Garczynski et al., 2005), and sNPFs (Garczynski et al., 2007) have been identified in *An. gambiae* while the GPCR receptor for a kinin has been identified from *A. stephensi* (Radford et al., 2004).

The likely GPCRs for other peptide hormones in mosquitoes were comparatively assessed by Vogel et al. (2013) using all of the Class A (245) and B (82) genes from *Ae. aegypti*, *An. gambiae*, *C. quinquefasciatus*, *D. melanogaster*, and *D. mojavensis*. This produced phylogenies comprised of well-supported monophyletic clades that were subdivided into assemblages and subassemblages. A majority of clades contained receptors whose peptide hormone ligands are known from experimental studies in *D. melanogaster* or other species, which in turn provided evidence for the likely ligand of corresponding orthologs in mosquitoes. These ‘characterized’ GPCR clades and their known or predicted peptide hormone ligands are listed in Table 2 using the nomenclature of Vogel et al. (2013). Data on where different receptors are expressed in mosquitoes and drosophilids are difficult to summarize because of inconsistencies between studies in regard to the life stages and tissues that were examined. We therefore discuss such expression data, if available in mosquitoes, in Section 4.1.

Vogel et al. (2013) also identified several GPCR ‘orphan’ clades. In some cases placement in the phylogeny provides insights into predicted ligands. For example, subassemblage 1a of the Class A GPCRs contains two orphan clades (OA1 and OA2) that are sister to the bursicon and glycoprotein A2/B5 receptors. This makes them strong candidates for binding relaxin/ILPs such as ILP6 in *Ae. aegypti* and ILP8 in *D. melanogaster* (Brown et al., 2008). Notably, this prediction was recently supported experimentally for *D. melanogaster* ILP8, which deorphanizes OA1 (Garelli et al., 2015;

Table 2 Peptide Hormone GPCR Genes for Three Mosquito Species and *D. melanogaster*^a

GPCR Class	Hormone	<i>Ae. aegypti</i>	<i>An. gambiae</i>	<i>C. quinquefasciatus</i>	<i>D. melanogaster</i>
A, Cluster 1					
1a	Bursicon	AAEL004777	AGAP008347	CPIJ011619	CG8930
1a	Glycoprotein A2/B5	AAEL004399	AGAP004035	CPIJ007712	CG7665
1a	Drosophila ILP8				CG31096
1b	CCAP R1	AAEL008652	AGAP001961	CPIJ006268	
1b	CCAP R2	AAEL008655	AGAP001962	CPIJ006269	CG33344
1b	Corazonin	AAEL011475	AGAP001558	CPIJ016679 CPIJ016678	CG10698
1b	ACP	AAEL009673	AGAP001532	CPIJ001199	
1b	AKH	AAEL011325	AGAP002156		CG11325
1b	Allatotropin	AAEL005310 AAEL011680		CPIJ014752	
2a	Sulfakinin R1	AAEL010207	AGAP001022	CPIJ005574	CG32540
2a	Sulfakinin R2			CPIJ016281 CPIJ016282	CG42301
2b	RYamide R1	AAEL008296	AGAP000351	CPIJ019394	CG5811
2b	RYamide R2	AAEL015418	AGAP000115	CPIJ018504	
2b	Tachykinin		AGAP002824	CPIJ014103 CPIJ007277	CG6515
2b	Natalisin	AAEL006947	AGAP001592		CG7887

2b	Kinin	AAEL011026 AAEL006636	AGAP010851	CPIJ012071	CG10626
2b	NPF	AAEL010626 NPYR2	AGAP004122-3	CPIJ006984 CPIJ018265	CG1147
2b	sNPF	AAEL013505 AAEL007924	AGAP012378	CPIJ13069	CG7395
2b	SIFamide	AAEL009698 AAEL005994	AGAP003335	CPIJ017622 CPIJ016970	CG10823
2d	CCH amide R1	AAEL016997	AGAP011452	CPIJ017639	CG30106
2d	CCH amide R2	AAEL017410	AGAP003631		CG14593
2d	ASTC R1	AAEL012920	AGAP010486	CPIJ011191	CG7285
2d	ASTC R2	AAEL012356	AGAP012268	CPIJ010469	CG13702
2d	ASTA R1	AAEL007169	AGAP003658	CPIJ016163	CG2872
2d	ASTA R2	AAEL006076-7	AGAP001773-4	CPIJ013095 CPIJ011118	CG10001
2e	Trissin	AAEL004732	AGAP008702	CPIJ001962-3	CG34381
2f	PK R1	AAEL017335 AAEL003747	AGAP003076	CPIJ011105-07	CG8784 CG8795D
2f	PK R2	AAEL012796 KC155994	AGAP000658	CPIJ014065 CPIJ019566 CPIJ015545	CG9918
2f	ETH R1	AAEL005803	AGAP002881	CPIJ003421	CG5911
2f	ETH R2	AAEL005804		CPIJ003422	

Continued

Table 2 Peptide Hormone GPCR Genes for Three Mosquito Species and *D. melanogaster*—cont'd

GPCR Class	Hormone	<i>Ae. aegypti</i>	<i>An. gambiae</i>	<i>C. quinquefasciatus</i>	<i>D. melanogaster</i>
A, Cluster 2					
	FMRFamide	AAEL015604 AAEL003378	AGAP001862	CPIJ007187	CG2114
	MIP	AAEL010313	AGAP012427	CPIJ007973	CG16752
	Myosuppressin	AAEL006283	AGAP005229	CPIJ000143	CG43745 CG8985
B					
	Pigment dispersing hormone	AAEL009024	AGAP003654	CPIJ009749	CG13758
	DH44 R1	AAEL008292	AGAP005464	CPIJ008822	CG12370
	DH44 R2	AAEL005894	AGAP005465	CPIJ008820	CG8422
	DH33	AAEL010043	AGAP009770	CPIJ014419	CG32843

³Gene entries for each species as in [Table 1](#). Receptor classification per [Vogel et al. \(2013\)](#). The Class A GPCR phylogeny forms two clusters (1 and 2). Cluster 1 is further divided into subassemblages 1a, b, and 2a–f.

Vallejo et al., 2015). Other orphan clades provide no insights about their ligands but their relationship to other GPCRs in the phylogeny strongly suggest they bind peptide hormones. If correct, this suggests that mosquitoes and drosophilids produce several peptide hormones that have not been identified. Lastly, these data show that dipteran Class A and B GPCRs have experienced several instances of duplication or loss in particular species, which results in some characterized clades containing ‘in group’ orphans (Vogel et al., 2013). The functional significance of this is unclear although duplicated receptors may preferentially bind different isoforms of a given peptide hormone since differential processing can produce variants of the same ligand (Predel et al., 2010). Recent studies also provide some support for this suggestion (Liesch et al., 2013).

2.2.2 PKRs

Most PKRs are single subunit receptors that have an extracellular ligand binding domain, a single hydrophobic transmembrane-spanning domain, and an intracellular kinase domain that upon ligand binding autophosphorylates either tyrosine or serine/threonine residues (Hubbard and Miller, 2007; Lemmon and Schlessinger, 2010). Most PKRs form noncovalent dimers upon entry into the plasma membrane or ligand binding, although some, like the insulin receptor (IR) (see later), form a covalent heterotetramer through intra- and intersubunit disulphide bonds. PKRs with tyrosine phosphorylation activity are referred to as receptor tyrosine kinases (RTKs) which can activate one or more signalling pathways. PKRs with serine/threonine kinase domains are also capable of activating one or more signalling pathways.

PKR ligands in mammals include insulin, several growth factors, and certain cell surface molecules (Lemmon and Schlessinger, 2010). A homologue of the vertebrate IR, was cloned from *D. melanogaster* prior to sequencing of its genome, which was named the *Drosophila* IR (Fernandez-Almonacid and Rosen, 1987). Biochemical studies showed this receptor binds vertebrate insulin (Fernandez-Almonacid and Rosen, 1987; Sajid et al., 2011) while genetic studies provided evidence that several endogenous ILPs activate the insulin signalling pathway (Brogiolo et al., 2001). Genetic studies in *D. melanogaster* also identified an RTK as the receptor for PTTH (Rewitz et al., 2009). In mosquitoes, experiments conducted in *Ae. aegypti* show that its IR binds endogenous ILP3 with high affinity which activates the canonical insulin signalling pathway (Brown et al., 2008; Dhara et al., 2013; Wen et al., 2010). In contrast, no functional

studies have been conducted in mosquitoes on the PTH receptor (see [Section 4.1](#)).

Results from [Vogel et al. \(2013\)](#) assemble the mosquito and drosophilid PKR genes into two branches: 12 well-supported clades consisting of RTKs and 5 clades containing PKRs with conserved serine–threonine kinase domains that are related to mammalian transforming growth factor beta (TGF- β) receptors. Unlike GPCRs, this analysis also shows that dipteran PKRs have undergone few lineage-specific gains or losses, which suggest the same genes are likely present in most or all Diptera. Mosquito and drosophilid IRs form a single clade that is closely related to a clade that contains homologues of human anaplastic lymphoma RTK (ALK) and leucocyte RTK plus a second orphan clade (OR1) lost from *D. melanogaster*. The ALK-like receptor has been shown to have functional roles in development, ethanol sensitivity, and learning in *D. melanogaster* but its ligand is unknown ([Gouzi et al., 2011](#); [Lasek et al., 2011](#); [Lorén et al., 2001](#)). The OR1 receptor from *Ae. aegypti* was recently deorphanized by showing that it binds OEH ([Vogel et al., 2015](#); see [Section 4.1](#)). Many of the remaining clades whose ligands are growth factors have been studied in *D. melanogaster* but have not been examined in mosquitoes. The RTK genes with known or predicted peptide hormone ligands in mosquitoes and *D. melanogaster* are summarized in [Table 3](#).

2.2.3 RGCs

RGCs are conserved homodimeric membrane proteins which have an extracellular ligand binding domain, a single pass transmembrane domain, and an intracellular catalytic domain with adenylyl or guanylyl cyclase activities that are sensitive to intracellular calcium levels ([Potter, 2011](#)). RGCs also usually form dimers prior to or after ligand binding. Studies in mammals were the first to show that RGCs bind peptide hormones called brain and atrial natriuretic peptides ([Potter, 2011](#)). In insects, experiments with the oriental fruitfly, *Bactrocera dorsalis*, identified an RGC as the receptor for eclosion hormone (EH) ([Chang et al., 2009](#)), while a second RGC was identified as the receptor for neuropeptide-like peptide 1 (NPLP1) in *D. melanogaster* ([Overend et al., 2012](#)). Phylogenetic analysis of mosquito and drosophilid RGCs generated one clade containing predicted EH receptors, a second containing NPLP receptors, and four orphan clades (OGC1–4) ([Vogel et al., 2013](#)) ([Table 3](#)). One of these orphan clades appears orthologous to the vertebrate RGCs that bind natriuretic peptides, while a

Table 3 Peptide Hormone RTK and RGC Genes for Three Mosquito Species and *D. melanogaster*^a

Receptor	Hormone	Ae. aegypti	An. gambiae	C. quinquefasciatus	D. melanogaster
RTK	ILPs	AAEL002317	AGAP012424	CPIJ006878	CG18402
RTK	ILPs	AAEL002317	AGAP012424	CPIJ006878	CG18402
RTK	PTTH	AAEL002404 AAEL010923	AGAP005763	CPIJ001844 CPIJ000058	CG1389
RGC	NPLPs	AAEL008390	AGAP012163	CPIJ010083	CG42636 CG42637
RGC	EH	AAEL008387	AGAP012161	CPIJ010082	CG10738

^aGene entries for each species as in [Table 1](#).

second appears orthologous to vertebrate retinal RGCs. The other two orphan clades have no vertebrate ortholog.



3. SOURCES, PROCESSING, AND RELEASE OF PEPTIDE HORMONES IN MOSQUITOES

In mosquitoes, most insights into where different peptide hormones are produced derive from studies of adult females. This bias mirrors the literature at-large where most of what we know about mosquito genetics, biochemistry, physiology, and behaviour also come from studies of adults, which is the life stage of interest from the perspective of vector biology and disease transmission. A different situation, however, exists in a number of other insects including *D. melanogaster* where the source of many peptide hormones have been carefully mapped in larvae but distribution in adults is less detailed (Nässel and Winther, 2010). Studies on peptide hormone production and function in Lepidoptera also overall focus on larvae because of the historic interest in these insects as models for the study of moulting (Nijhout, 1998; Smith and Rybczynski, 2012; Truman and Riddiford, 2002). Thus, while most peptide hormone genes in mosquitoes are conserved with other insects, differences in functional priorities prevent some comparisons from being made in regard to where they are produced. The emphasis on adults has also affected which peptide hormones have been most studied in mosquitoes.

3.1 Peptide Hormone Producing Cells

Immunocytochemistry and peptidomics provide information on cells in mosquitoes where different peptide hormones have been detected. We list the species for which such data are available in Table 1 and illustrate these sites in Fig. 1. For the central nervous system several hormones are detected in the brain, specific neurosecretory cells, and ganglia of the ventral nerve chord (Brown and Cao, 2001; Brown and Lea, 1988; Brown et al., 2008; Cao and Brown, 2001; Estévez-Lao et al., 2013; Hellmich et al., 2014; Hernández-Martínez et al., 2005; Kaufmann and Brown, 2006; Kaufmann et al., 2009; Krieger et al., 2004; Marquez et al., 2011; Meola and Lea, 1972; Meola et al., 1998; Moffett and Moffett, 2005; Predel et al., 2010; Riehle et al., 2006; Siju et al., 2013; Stracker et al., 2002). The corpus cardiacum (CC) and perivisceral organs iteratively present in abdominal segments are neurohemal organs that store and release several different peptide hormones in mosquitoes (Brown and Cao, 2001; Brown and

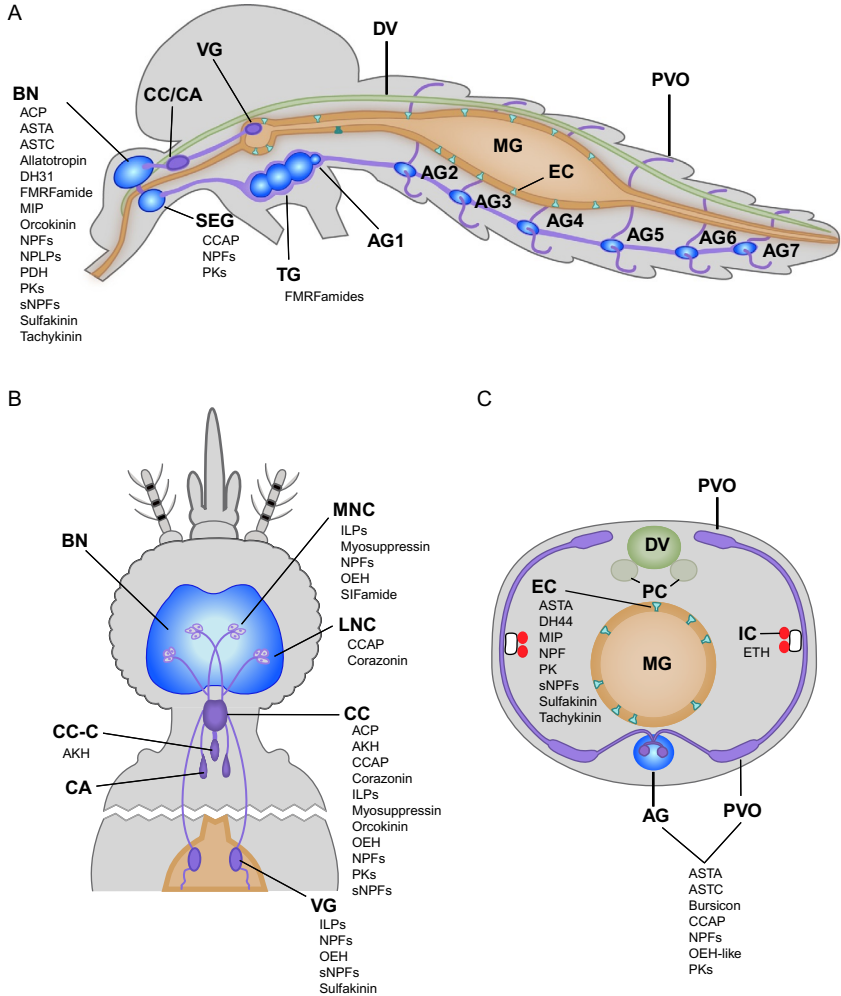


Fig. 1 Schematic illustrating the sources of different peptide hormones in adult female mosquitoes as determined by immunocytochemistry or peptidomics. Sagittal view of the whole body (A), dorsal view of the head and thoracic regions (B), and a cross section through the third abdominal segment (C). Select regions of the nervous system, organs and cells are labelled in bold. Peptide hormones in [Table 1](#) are listed alphabetically below detected sites. Abbreviations: *AG1–7*, abdominal ganglia 1 through 7; *BN*, brain; *CA*, corpora allata; *CC*, corpus cardiacum; *CC-C*, corpus cardiacum intrinsic cells; *DV*, dorsal vessel; *EC*, gut endocrine cells; *IC*, Inka cells; *LNC*, lateral neurosecretory cells; *MG*, midgut; *MNC*, medial neurosecretory cells; *PC*, pericardia; *PVO*, perivisceral organs; *SEG*, subesophageal ganglion; *VG*, ventricular ganglion. Peptide hormone abbreviations are listed in [Table 1](#). Most of these data derive from studies of adults with the exception being for ETH, which is shown in (C) although data derive from studies of larvae (see text).

Lea, 1988; Cao and Brown, 2001; Estévez-Lao et al., 2013; Hernández-Martínez et al., 2005; Honegger et al., 2011; Kaufmann and Brown, 2006; Kaufmann et al., 2009; Marquez et al., 2011; Predel et al., 2010; Riehle et al., 2006; Veenstra and Costes, 1999). Studies conducted primarily in *D. melanogaster* show that the midgut epithelium contains intestinal stem cells, which self-renew and differentiate into two other cell types: enterocytes, that produce digestive enzymes and mediate nutrient uptake, and endocrine cells (Jiang and Edgar, 2012). In *Ae. aegypti*, midgut endocrine cells are known to produce and/or release several peptide hormones (Brown et al., 1986; Hernández-Martínez et al., 2005; Marquez et al., 2011; Moffett and Moffett, 2005; Predel et al., 2010; Stracker et al., 2002; Veenstra et al., 1995) (Table 1, Fig. 1).

3.2 Processing Enzymes and Hormone Secretion

Microscopy studies show that peptide hormones accumulate in the body of endocrine and neurosecretory cells where these are packaged into secretory vesicles as propeptides along with processing enzymes (Brown and Lea, 1988; Raabe, 1989). Studies of mammalian peptidergic cells indicate these include endopeptidases, which are primarily convertases that process propeptides, and other modifying enzymes that can alter the amino- and carboxyl termini of processed peptides in ways that are important for function (Hook et al., 2008; Isaac et al., 2009). Amidation of carboxyl termini also confers resistance to peptidases, which potentially extends half-life after release into circulation. Functional studies of convertases and the enzymes responsible for carboxy-terminal amidation have been conducted in *D. melanogaster* (Jiang et al., 2000; Reiher et al., 2011; Taghert and Veenstra, 2003; Wegener et al., 2011). Genes encoding homologous processing enzymes have been identified in mosquitoes but no functional studies have been conducted (Akbari et al., 2013; Matthews et al., 2016; Riehle et al., 2002).

Peptide hormones are released from endocrine and neurosecretory cells in response to external and internal stimuli. For neurosecretory cells in the brain, this release occurs primarily from axons extending to the CC and along the gut (Brown and Cao, 2001; Cao and Brown, 2001; Raabe, 1989). In contrast, neurosecretory cells located in other ganglia or tissues release peptide hormones in several other locations throughout the body (Raabe, 1989). Midgut endocrine cells release peptides into the space between epithelial cells for diffusion to neighbouring cells and the

hemolymph (Brown et al., 1986, 1988). Several enzymes that degrade peptide hormones in circulation or after binding to target cells have been identified in mammals (Isaac et al., 2009). While homologues of these enzymes are known in both mosquitoes and *D. melanogaster* (Riehle et al., 2002; Taghert and Veenstra, 2003), little information is available regarding their expression or function. The only exception to this is in the context of data showing that some kinin agonists are resistant to proteolysis in mosquitoes, which suggests they could be useful as control agents (Taneja-Bageshwar et al., 2009).

Some peptide hormones are also likely degraded by endosomal enzymes after receptor binding and internalization. One example of this is insulin degrading enzymes (IDE, insulinases) that are conserved between mammals and insects including *An. gambiae* and *D. melanogaster* (Duckworth et al., 1989; Riehle et al., 2002). A recent study showed that an IDE is globally expressed in *D. melanogaster* throughout development, is present in both the membrane and cytoplasm of cells, and affects ILP levels and signalling (Galagovsky et al., 2014). Studies from mammals also suggest IDEs may have important roles in intracellular degradation of other peptides (Fernández-Gamba et al., 2009).



4. PEPTIDE HORMONE FUNCTIONS IN MOSQUITOES

Many studies have been conducted on the functions of peptide hormones in insects, but this literature is also large and difficult to summarize across species that have been studied. As a result, most reviews focus on either: (1) processes that peptide hormones have roles in regulating like metabolism (Baker and Thummel, 2007; Broughton and Partridge, 2009), moulting and metamorphosis (Smith and Rybczynski, 2012; White and Ewer, 2014), and water balance (Dow and Davies, 2006) or (2) particular types of peptide hormones such as FMRFamides (Nichols, 2003), ILPs (Antonova et al., 2012), or PRXamides (Jurenka, 2015). A few recent summaries discuss peptide hormone functions more broadly but they primarily emphasize nonmosquito species, which results in a number of studies from the mosquito literature being uncited (Hauser et al., 2008; Nässel and Winther, 2010; Verlinden et al., 2014). Our own assessment identifies functional data in one or more mosquito species for about a third of the entries in Table 1. Below, we first summarize these findings in alphabetical order with the absence of a given peptide from Table 1 indicating that no functional studies to our knowledge have been conducted in

mosquitoes. We also provide a succinct summary of comparative information, so that the reader can put into firmer context findings from mosquitoes. We then follow this information with some concluding remarks.

4.1 Peptide Hormones with Reported Activities

4.1.1 Adipokinetic Hormone (AKH) and AKH/Corazonin Peptide (ACP)

Most insects including *D. melanogaster* have one AKH gene that is processed into an N-terminally pyroglutamate blocked peptide of 8–10 amino acids (Gäde, 2009). Functional studies outside of mosquitoes report that AKHs mobilize stored lipid and/or carbohydrate from the fat body into the hemolymph; making this hormone a functional analogue of mammalian glucagon (Van der Host, 2003). Studies from *D. melanogaster* focus primarily on AKH function in concert with ILPs in regulating metabolism (Baker and Thummel, 2007; Nässel and Winther, 2010) although one study also reports that AKH is cardiostimulatory (Noyes et al., 1995). As noted earlier, mosquitoes have AKH and ACP genes, which give rise to two peptides that bind class A GPCRs in subassemblage 1b. These receptors are also related to the receptors for CCAP and allatotropin (Belmont et al., 2006; Caers et al., 2012; Mugumbate et al., 2011, 2013; Vogel et al., 2013). Functional experiments indicate that AKH mobilizes stored carbohydrate but not lipid in *An. gambiae* while ACP has no effect on either lipid or carbohydrate mobilization (Kaufmann and Brown, 2008).

4.1.2 Allatostatin A and C (ASTA and C)

ASTs are structurally variable peptides from distinctly different genes (Veenstra, 2008). The first AST was identified from the cockroach *Diploptera punctata* as an allatostatin (Woodhead et al., 1989). However, studies report activities for ASTs in other insects that do not include the inhibition of juvenile hormone (JH). Thus, the name AST for these peptides is largely historical. We thus include allatostatin B (ASTB) with the myoinhibitory peptides (MIP) on the basis of sequence similarity (Table 1). In mosquitoes and drosophilids, ASTA and C bind related class A GPCRs that cluster together into subassemblage 2c along with the receptor for CCHamide (Mayoral et al., 2010; Vogel et al., 2013). ASTA and C have both been shown to regulate JH biosynthesis in *Ae. aegypti* (Li et al., 2004, 2006), while one report suggests a role for ASTs in gut motility (Onken et al., 2004). The role of ASTs in regulation of JH is comprehensively discussed in chapter “The Role of Juvenile Hormone in Mosquito Development and Reproduction” by Zhu and Noriega.

4.1.3 Allatotropin

Allatotropins are 14 amino acid peptides that stimulate JH biosynthesis in insects from several orders. As previously noted, however, drosophilids lack orthologous genes for both allatotropin and its receptor (Vogel et al., 2013). Functional studies identify the Class A, subassemblage 1a GPCR that binds allatotropin in *Ae. aegypti* while also demonstrating that allatotropin stimulates JH biosynthesis (Hernández-Martínez et al., 2007; Li et al., 2003; Nouzova et al., 2012). These results are further discussed in chapter “The Role of Juvenile Hormone in Mosquito Development and Reproduction” by Zhu and Noriega. Vogel et al. (2013) identified a closely related paralog of the allatotropin receptor in *Ae. aegypti* but its ligand is unknown.

4.1.4 Bursicon and Partner of Bursicon

Bursicon has long been known in the literature as a factor required for tanning of insect cuticle after moulting (Nijhout, 1998). However, it was only recently identified in *D. melanogaster* as a 30 kDa heterodimer that is produced from products of the bursicon and partner of bursicon genes (Luo et al., 2005; Mendive et al., 2005). The Class A GPCR that binds bursicon in *D. melanogaster* clusters in subassemblage 1a with single orthologs in all mosquitoes (Vogel et al., 2013). This subassemblage also contains receptors that bind biogenic amines, steroid hormones, and certain large peptide ligands. As such, subassemblage 1a receptors are referred to as derived-peptide GPCRs (Vogel et al., 2013). The adult bias of the literature largely underlies the paucity of data on bursicon function in mosquitoes although one study does show that bursicon-producing neurosecretory cells undergo apoptosis in *An. gambiae* after adult ecdysis (Honegger et al., 2011).

4.1.5 Corazonin

An 11 amino acid peptide structurally similar to AKHs was isolated and named corazonin based on its activation of heart contractions in the cockroach *Periplaneta americana* (Veenstra, 1989). Characterization of the corazonin gene in mosquitoes and other insects revealed this similarity extended to their prepropeptides and led to the identification of a gene encoding another peptide named ACP (Hansen et al., 2010). These prepropeptides show structural similarity to the gonadotropin-releasing hormones (GnRH) in mammals. AKH, corazonin, and ACP bind different but closely related Class A GPCRs in *An. gambiae* that belong to subassemblage 1b (Belmont et al., 2006; Hansen et al., 2010; Vogel et al., 2013). These GPCRs also share homology with the GnRH GPCRs

(Hauser and Grimmelikhuijzen, 2014). The function and expression of ACP and its receptor are unknown in insects, whereas corazonin has a range of reported activities including being cardioactive (Hauser and Grimmelikhuijzen, 2014). However, studies in *An. gambiae* indicate no significant effect on dorsal vessel contraction after knockdown of this factor and its receptor by RNA interference (RNAi) (Hillyer et al., 2012).

4.1.6 Crustacean Cardioactive Peptide (CCAP)

CCAPs are cyclic, amidated nine amino acid peptides that were first identified from the crab *Carcinus maenas* but are widely conserved regulators of dorsal vessel contraction in insects and other arthropods (Stangier et al., 1999; White and Ewer, 2014). CCAP has also been implicated in regulating ecdysis (White and Ewer, 2014). The Class A, subassemblage 1b GPCR that binds CCAP was first identified in *D. melanogaster* and later in *An. gambiae* (Belmont et al., 2006). Expression studies in adult *An. gambiae* establish that CCAP localizes to brain neurosecretory cells with axons to the CC, and cells in abdominal ganglia with axons to the heart (Estévez-Lao et al., 2013) (Fig. 1). CCAP injected into adult female *An. gambiae* stimulates heart contractions, whereas RNAi knockdown of CCAP had the opposite effect (Chen and Hillyer, 2013; Estévez-Lao et al., 2013). Both water and sucrose deprivation reduce heart contraction rate but these effects occur independently of CCAP activity (Ellison et al., 2015).

4.1.7 Diuretic Hormone 31 (DH31, Calcitonin-Like)

This hormone was first identified from the cockroach *Di. punctata* as a 31 amino acid peptide with a C-terminal GXPamide that shares structural features and diuretic activity with vertebrate calcitonins (Furuya et al., 2000). DH31 homologues are now known from several other insects (Zandawala, 2012). Early studies showing that a peptide hormone regulates diuresis in mosquitoes after blood feeding (see earlier) was identified as a DH31 homologue in *An. gambiae* (Coast et al., 2005). Identification of the DH31 receptor in *D. melanogaster* as a Class B GPCR was followed by identification of its ortholog in *Ae. aegypti*, which is expressed as a gradient along the Malpighian tubules (Kwon et al., 2012). Expression in the hindgut further led to the discovery that DH31 exhibits myotropic activity (Kwon and Pietrantonio, 2013). These data are further elaborated upon in chapter “Renal Excretory Processes in Mosquitoes” by Piermarini.

4.1.8 Diuretic Hormone 44 (Corticotropin-Releasing Factor-Like)

A 41 amino acid peptide with diuretic activity that was initially identified from the moth *Manduca sexta* with its receptor (Kataoka et al., 1989; Reagan, 1994). DH44 sequences exhibit similarities to peptide hormones in the vertebrate corticotropin-releasing factor (CRF) family that bind Class B GPCRs (Balment and Lovejoy, 1999; Cardoso et al., 2014). A DH44 homologue was isolated from whole body extracts of *Culex salinarius* that increased cAMP levels in *M. sexta* Malpighian tubules and stimulated diuresis in *Ae. aegypti* tubules (Clark et al., 1998). Subsequently, two Class B GPCRs that bind DH44 were identified from *D. melanogaster* while one was identified from *Ae. aegypti* (Hector et al., 2009; Jagge and Pietrantonio, 2008). More information on DH44 is provided in chapter “Renal Excretory Processes in Mosquitoes” by Piermarini.

4.1.9 Ecdysis Triggering Hormone (ETH)

Studies conducted in *M. sexta* identified ETH as a 26 amino acid peptide that ended at its C-terminus with the sequence PRMamide (Zitnan et al., 1996). ETH genes encoding usually two ETH peptides have since been identified from insects in most orders including Diptera (Jurenka, 2015; Zitnan et al., 2003). As noted earlier, ETH peptides are conservatively produced in Inka (=epitracheal) cells (Fig. 1). ETH genes and the mature peptides they encode exhibit distinct features. However, the C-terminal PRXamide motif of ETHs is shared with another group of peptide hormones we refer to below as pyrokinins (see Section 4.1.15). ETHs also bind Class A GPCRs that are closely related to the pyrokinin receptors (Vogel et al., 2013). In *D. melanogaster* the ETH receptor gene is alternatively spliced to produce receptors that preferentially bind the two mature ETHs that are processed from prepro-ETH (Iversen et al., 2002; Park et al., 2002). Mosquito ETH genes also produce two ETH isoforms but no studies have examined receptor binding. Functional data support a conserved role for ETH in triggering ecdysis in *Ae. aegypti* during larval and pupal moulting (Dai and Adams, 2009). A recent study also implicates ETH in the timing of JH synthesis (Areiza et al., 2014).

4.1.10 FMRFamides

These neuropeptides are 7–10 amino acids in length, share C-terminal RFamide sequences, and have broadly conserved myotropic functions. The literature initially treated peptides ending in RF/Pamide including

insect FMRFamides, sulfakinins, myosuppressins, NPF, and sNPF as related 'FMRFamide' or 'FaRPamide' peptides. These peptides also all bind Class A GPCRs. Subsequent studies, however, showed that FMRFamides, sulfakinins, myosuppressins, NPFs, and sNPFs derive from distinctly different genes, which suggests their similar C-termini reflects evolutionary convergence driven by currently unknown factors. The receptors for mosquito and drosophilid FMRFamides all reside in a single cluster 2 clade (Vogel et al., 2013). The myotropic action of FMRFamides on the heart and gut of *D. melanogaster* as well as their expression in the nervous system are well studied (Nichols, 2003). The first functional studies in mosquitoes used an FMRFamide antiserum to identify FMRFamide cells that were immunopositive in the nervous system and midgut of *Ae. aegypti* (Brown and Lea, 1988; Brown et al., 1986; Moffett and Moffett, 2005). These data though provide unclear results regarding which of these cells actually produce and/or store FMRFamides because the antisera used recognizes only the RFamide epitope. Studies in *An. gambiae* show that a single dose of FMRFamide stimulates larval heart contractions (Dutlinger et al., 2003). Hillyer et al. (2014) profiled FMRFamide gene expression in *An. gambiae* and predicted prepro-FMRFamide produces eight FMRFamide peptides, which is consistent with previously discussed peptidomic data showing that prepro-FMRFamide from *Ae. aegypti* produces nine peptides (Predel et al., 2010). Functional experiments indicate that two FMRFamide peptides increase heart contractions in *An. gambiae* at low doses but reduce contractions at high doses (Hillyer et al., 2014). A direction for study that would aid in interpreting the physiological importance of FMRFamides in mosquito physiology would be to clearly identify the cell sources for these peptides and release dynamics.

4.1.11 Insulin-Like Peptides (ILPs)

All ILPs are 6–8 kDa, share a common structural motif called the insulin fold, and are processed from precursors with similar domain structure (Pre, B, C, A) (De Meyts et al., 2009). ILPs are also broadly conserved among metazoans and are the most studied peptide hormones because of their important regulatory roles in metabolism, growth, and development. In mammals, insulin is the only ILP that binds with high affinity to the IR; an RTK, which activates the insulin signalling pathway (De Meyts et al., 2009). Mammals also produce two other types of ILPs: (1) insulin-like growth factors (IGFs) that preferentially bind another RTK, the IGF receptor (IGFR), and activate MAPK signalling and (2) relaxins that bind GPCRs and activate

several signalling pathways (Bani, 1997; McDonald et al., 1989). Insulin primarily regulates metabolic responses, IGFs primarily have functions in growth, while relaxins are implicated in several activities.

This background is important because mosquitoes and other insects produce multiple ILPs but usually have only one IR/IGFR homologue which as previously noted is named the IR. Several but not all ILP family members are functionally redundant in *D. melanogaster* but it is currently unknown whether all ILP family members are capable of binding and activating the IR (Grönke et al., 2010; Zhang et al., 2009). That ILP8 requires a GPCR for function suggests it is relaxin-like and may not interact with the IR (Garelli et al., 2015; see earlier). Factors released from the fat body plus the peptide hormone CCHamide2 from gut endocrine cells are both implicated in regulating ILP release from brain medial neurosecretory cells in *D. melanogaster* (Sano et al., 2015).

Studies in mosquitoes have mostly been conducted in *Ae. aegypti* where five ILP genes (1, 3, 4, 7, and 8) are expressed in brain medial neurosecretory cells while three (2, 5, and 6) are detected in other tissues including the midgut and fat body (Riehle et al., 2006). Each prepro-ILP exhibits features consistent with processing like vertebrate insulin/relaxins except ILP6, which has features that suggest it is processed like a vertebrate IGF (Brown et al., 2008; Riehle et al., 2006). Most functional data focus on ILP3 because it is most similar to vertebrate insulin, binds with high affinity to the *Ae. aegypti* IR, and activates the insulin signalling pathway (Brown et al., 2008; Dhara et al., 2013; Wen et al., 2010).

ILP3 exhibits vertebrate insulin-like activity by reducing blood sugar (trehalose) in adult female *Ae. aegypti* while elevating carbohydrate and lipid stores in the fat body (Brown et al., 2008). ILP3 also has several functions in female reproduction. As noted earlier, the mosquito endocrinology literature began with characterization of EDNH, which was renamed OEH. In the process of identifying OEH, data suggested factors smaller than OEH were released from the brain after blood feeding that had similar activity (Brown et al., 1998; Matsumoto et al., 1989a). Subsequent experiments demonstrated this factor was ILP3 which is released with OEH from medial neurosecretory cells (Fig. 1). The factors that regulate ILP and OEH release after blood feeding are unknown but data strongly support that ILP3 directly stimulates ovary follicle cells to produce ecdysone by binding the IR (Brown et al., 2008; Riehle et al., 2002; Wen et al., 2010). ILP3 also stimulates late-phase trypsin expression by the midgut, which is required for blood meal digestion, and proliferation of hemocytes (Castillo et al., 2011;

Gulia–Nuss et al., 2011). Other factors involved in regulation of egg formation are discussed in chapter “[Regulation of Reproductive Processes in Female Mosquitoes](#)” by Roy et al.

Functional studies of ILPs in other mosquitoes are restricted two lines of investigation. First, data support a role for insulin signalling in arrest of ovary development and lipid storage in overwintering diapause by *C. pipiens* (Sim and Denlinger, 2009). Second, ingestion of vertebrate insulin in a blood meal activates insulin signalling and synthesis of reactive oxygen species in the midgut of anopheline mosquitoes (Kang et al., 2008).

4.1.12 Kinin

The first kinin was identified from the cockroach *Rhyarobia* (formerly *Leucophaea*) *maderae* (Holman et al., 1986), which was followed by identification of structurally similar peptides from other insects (Hayes et al., 1997; Hewes and Taghert, 2001). The single kinin gene in *Ae. aegypti* encodes a prepropeptide that is processed into three isoforms but the kinin gene from *D. melanogaster* is processed into only one (Hewes and Taghert, 2001; Veenstra et al., 1997b). In turn, *Ae. aegypti* encodes two class A GPCRs that bind kinins while *D. melanogaster* encodes one (Hewes and Taghert, 2001; Pietrantonio et al., 2005; Taneja–Bageshwar et al., 2009; Vogel et al., 2013). Immunostaining localizes the kinin receptor to the membrane of endocrine cells in the posterior midgut, Malpighian tubules, and the rectal pads of female *Ae. aegypti* (Kersch and Pietrantonio, 2011). Vogel et al. (2013) assigns these receptors to subassemblage 2b along with the receptors that bind RYamide, tachykinin, NPF, sNPF, and SIFamide. Functional studies in *Ae. aegypti* provide strong support for the role of kinins in diuresis (Beyenbach and Piermarini, 2010; Kersch and Pietrantonio, 2011), which is further discussed in chapter “[Renal Excretory Processes in Mosquitoes](#)” by Piermarini.

4.1.13 Neuropeptide F (NPF)

NPFs are 28–40 amino acid peptides that share a consensus C-terminal RxRFamide motif (Nässel and Wegener, 2011). They are broadly conserved among invertebrates and also share homology with vertebrate neuropeptide Y (NPY) and pancreatic polypeptides that regulate appetite, digestion, reproduction, stress, and other activities (Holzer et al., 2012). The first insect NPF was identified from *D. melanogaster* on the basis of its RFamide immunoreactivity (Brown et al., 1999), which was followed by cloning of an NPF gene from *Ae. aegypti* (Stanek et al., 2002). Drosophilids and mosquitoes both encode a single NPF gene while mature NPFs bind

Class A GPCRs in subassemblage 2b that are most closely related to the receptors that bind sNPFs (Nüssel and Wegener, 2011; Vogel et al., 2013). This finding is interesting because NPFs and sNPFs are structurally distinct and derive from unrelated genes (see below). Functional studies in *D. melanogaster* indicate NPF has similar activities to NPY in mammals (Nüssel and Wegener, 2011). In contrast, little is known about NPF function in mosquitoes. Expression studies detect NPF mRNA in the brain and midgut of *Ae. aegypti* while binding studies show that mature NPF from *Ae. aegypti* and *An. gambiae* both bind the *An. gambiae* NPF receptor (Garczynski et al., 2005; Stanek et al., 2002). More recently, studies of five Class A, subassemblage 2 GPCRs from *Ae. aegypti* identified two that bind *Ae. aegypti* NPF (Liesch et al., 2013). The only reported function for NPF in mosquitoes is the inhibition of anterior midgut peristalsis in larval stage *Ae. aegypti* (Onken et al., 2004), but it is highly likely these peptide hormones have other, more functionally significant activities, given the greater literature.

4.1.14 Ovary Ecdysteroidogenic Hormone (OEH)

OEH is a relatively large 9–13 kDa hormone that as previously noted was identified from *Ae. aegypti* (Brown et al., 1998). It is structurally related to neuroparsins which are peptides of unclear function that were first isolated from locusts (Badisco et al., 2007). OEH is released with ILPs from medial neurosecretory cells with both stimulating ovary follicle cells to produce ecdysone (Fig. 1). Functional redundancy with ILPs together with the absence of an identified receptor obscured the mode of action of OEH for many years. This changed with identification of the OEH receptor as an RTK that is closely related to the IR (Vogel et al., 2015). Results also show that OEH directly induces ecdysone production by activating PI3K/Akt signalling (Dhara et al., 2013; Vogel et al., 2015). Thus, redundancy is due to OEH and ILPs binding different receptors on follicle cells that appear to both activate insulin pathway signalling. In contrast, OEH does not activate late-phase trypsin expression or haemocyte proliferation because OEH receptor expression is restricted to ovary follicle cells (Vogel et al., 2015). Some mosquito species have evolved from blood feeding relatives to produce eggs without blood feeding (autogeny). Recent studies show that facultatively autogenous *Georgacraigius* (= *Aedes*) *atropalpus* releases OEH and ILP from medial neurosecretory cells shortly after adult emergence but OEH is the primary regulator of ecdysone synthesis by the ovaries (Gulia-Nuss et al., 2012). OEH alone also stimulates limited egg development in nonblood fed *Ae. aegypti* (Gulia-Nuss et al., 2015).

These findings suggest the evolution of autogeny is due in part to the shift from blood meal dependent to blood meal-independent release of OEH and ILPs from the nervous system.

4.1.15 Pyrokinins (PKs)

Peptide hormones we name PKs are relatively small and usually end with the C-terminal motif PRL/Vamide. This motif is similar to that of ETHs discussed earlier, which is why these peptides are often referred to collectively as PRXamides (Jurenka, 2015). The PRXamide literature is very difficult to follow for most individuals. One reason is because of the range of activities PRXamide peptides exhibit. The other is confusing nomenclature. PRXamides are named for either the biological activity or structural features exhibited by the first peptides that were identified. Thus, cardioacceleratory peptide (CAPA) was the name given for a PRXamide purified from *M. sexta*, leucopyrokinin (PK), and perviscerokinin (PVK) for myotropic PRXamides from cockroaches (*R. maderae* and *P. americana*), diapause hormone for PRXamides from *Bombyx mori* and *Helicoverpa zea* (DH-2), ETHs for PRXamides from *M. sexta*, pheromone biosynthesis activating peptide (PBAN) for a PRXamide from *H. zea*, and diuretic hormone (DH-1) for a PRXamide from *P. americana* (see Jurenka, 2015 for primary citations).

Peptide hormones are often named this way. It also works well for ETHs, which derive from a single gene and exhibit conservation in sequence and function. In contrast, the other PRXamides often exhibit cross-reactivity or activities that differ from given names when studied in different species. They also derive from two distinct genes. The first of these was named *pban* in *H. zea* because it encoded the corresponding peptide. However, *pban* from *H. zea* also contains four other PRXamides: DH-2 and three others referred to as PKs (Jurenka, 2015). Orthologs of this gene are known in many insects including *D. melanogaster* where it is named *hugin*. *Hugin* though encodes only two PRXamides named PK and *hugin* λ (see Nässel and Winther, 2010). The second gene is *capability* (*capa*), which was first identified in *D. melanogaster* with orthologs also now known from other insects. This gene usually contains three PRXamides: two named CAPA or CAPA-PVK peptides and a third named DH-1 or PK depending on publication (see Jurenka, 2015; Nässel and Winther, 2010). These names derive from sequence similarities with the originally identified CAPA, PVK, or PKs but again functional data often differ when studied in different species.

Given this background, we discuss ETHs as distinct peptide hormones (see earlier) because their structure and function support this. In contrast,

the functional literature does not currently support giving different names to the peptides encoded by the *pban/hugin* or *capa* genes. We therefore name these genes in mosquitoes *pk1* and *pk2*. In *Ae. aegypti* *pk1* encodes four peptides we name PK1a–d while *pk2* encodes three we name PK2a–c. The Class A GPCRs in mosquitoes and drosophilids that bind peptides encoded by the above genes form one clade in subassemblage 2F while the receptors that bind ETHs form a sister group (Choi et al., 2013; Dai and Adams, 2009; Olsen et al., 2007; Vogel et al., 2013). Peptidomic and immunocytochemistry methods detect PKs in the CNS, periviseral organs, CC, and midgut of adult mosquitoes (Hellmich et al., 2014; Predel et al., 2010). The only functional data in mosquitoes show that PK2a from *Ae. aegypti* inhibits fluid secretion in larval Malpighian tubules at femtomolar concentration but stimulates diuresis at higher concentrations (Ionescu and Donini, 2012). By previous nomenclature PK2a would be referred to as CAPA1 (Jurenka, 2015), which is inconsistent with its diuretic activity.

4.1.16 Short Neuropeptide F (sNPF)

sNPFs are peptides of variable length (6–19 amino acids) that end with the consensus C-terminal motif PxLRLRFamide in insects (Nässel and Wegener, 2011). All sequenced insect and other invertebrate genomes contain one or more sNPF genes but orthologs are absent from vertebrates. Among holometabolous insects like mosquitoes, prepropeptides often contain multiple sNPF sequences, whereas hemimetabolous insects and other invertebrates tend to produce prepropeptides that contain only one sNPF. The naming of NPFs and sNPFs derives from early studies in the literature where multiple peptides with related C-termini were identified. Later studies showed that NPFs and sNPFs derive from different genes and bind distinct but related class A, GPCRs that for Diptera reside in subassemblage 2b (Nässel and Wegener, 2011; Vogel et al., 2013). We earlier noted that the sNPF gene has duplicated in *Ae. aegypti* but not in *An. gambiae* or *C. quinquefasciatus*. *Ae. aegypti* sNPF1 encodes what was historically named sNPF while sNPF2 contains three copies of what was originally named head peptide (Matsumoto et al., 1989b; Stracker et al., 2002).

A diversity of neurons in the CNS and chemosensory structures like the antennae express sNPF in *D. melanogaster* while functional data implicate sNPFs in regulating feeding, growth, and ILP production by medial neurosecretory cells (Nässel and Winther, 2010). The literature likewise suggests sNPFs are broadly expressed in the adult mosquito CNS (Garczynski et al., 2007; Matsumoto et al., 1989b; Predel et al., 2010; Siju et al., 2013; Stracker

et al., 2002; Veenstra, 1999) (Fig. 1), while a recent study reports male accessory glands in *Ae. aegypti* produce and transfer sNPF2 to females during mating (Naccarati et al., 2012). Early functional experiments in *Ae. aegypti* showed that sNPF2 titer increases in the hemolymph of adult females after consuming a blood meal, which coincides with females exhibiting no host-seeking behaviour (Brown et al., 1994). Nonblood fed females in contrast are highly responsive to vertebrate hosts but injection of sNPF2 inhibited host seeking for up to 5 h which suggested a role for sNPF2 in regulating host-seeking behaviour (Brown et al., 1994). More recent studies confirm that *Ae. aegypti* NPF and sNPFs bind different receptors but also detected overlap in receptor binding between sNPF1 and sNPF2 (Liesch et al., 2013). Bioassays identified roles for both NPF and sNPFs in host-seeking behaviour by nonblood females, yet curiously mutagenesis of the GPCR that binds sNPFs has no effect on host seeking, sugar, and blood-feeding behaviour, egg laying, or locomotion (Liesch et al., 2013). Naccarati et al. (2012) tested whether sNPF2 regulates female mating receptivity in *Ae. aegypti* because a structurally unrelated factor named sex peptide in male accessory gland secretions of *D. melanogaster* exhibits this activity. However, sNPF2 had no effect on female mating receptivity. Lastly, one study reports that sNPF1 and two both inhibit peristalsis of the *Ae. aegypti* larval anterior midgut (Onken et al., 2004).

4.2 Concluding Remarks

Significant progress has been made in identifying peptide hormone genes and receptors in mosquitoes. The functional literature is also strong in a few areas of study that focus on adult females. These include the roles of peptide hormones in regulating egg formation, JH biosynthesis, and diuresis, which are also subjects of separate chapters in this volume. In contrast, mapping the cell sources of different peptide hormones across life stages remains weak as is the literature on functional activities during immature development. For example, the endocrine regulation of moulting and metamorphosis in *D. melanogaster* implicates PTTH in regulating ecdysone production by prothoracic glands (PGs) and insulin signalling in nutrition-dependent growth (Nijhout et al., 2014; Rewitz et al., 2009). No data, however, support that PTTH or ILPs directly activate ecdysone production (Niwa and Niwa, 2014). Given the literature on many other insects, it is somewhat ironic that surprisingly little is known about the regulation of moulting in mosquitoes. An early study showed that larval thoracic and abdominal wall tissues produce ecdysone yet also reported that PGs do not

(Jenkins et al., 1992). These findings together with the *Drosophila* literature indicate our understanding of peptide hormone functions in moulting are far from complete for Diptera. The orphan GPCR, RTK, and RGCs in mosquitoes also suggests some peptide hormones remain to be identified and characterized.

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