Chapter 8
Molecular Basis of Pheromonogenesis Regulation in Moths

J. Joe Hull and Adrien Fónagy

Abstract Sexual communication among the vast majority of moths typically involves the synthesis and release of species-specific, multicomponent blends of sex pheromones (types of insect semiochemicals) by females. These compounds are then interpreted by conspecific males as olfactory cues regarding female reproductive readiness and assist in pinpointing the spatial location of emitting females. Studies by multiple groups using different model systems have shown that most sex pheromones are synthesized *de novo* from acetyl-CoA by functionally specialized cells that comprise the pheromone gland. Although significant progress was made in identifying pheromone components and elucidating their biosynthetic pathways, it wasn't until the advent of modern molecular approaches and the increased availability of genetic resources that a more complete understanding of the molecular basis underlying pheromonogenesis was developed. Pheromonogenesis is regulated by a neuropeptide termed Pheromone Biosynthesis Activating Neuropeptide (PBAN) that acts on a G protein-coupled receptor expressed at the surface of pheromone gland cells. Activation of the PBAN receptor (PBANR) triggers a signal transduction cascade that utilizes an influx of extracellular Ca^{2+} to drive the concerted action of multiple enzymatic steps (i.e. chain-shortening, desaturation, and fatty acyl reduction) that generate the multicomponent pheromone blends specific to each species.

In this chapter, we provide a brief overview of moth sex pheromones before expanding on the molecular mechanisms regulating pheromonogenesis, and conclude by highlighting recent developments in the literature that disrupt/exploit this critical pathway.

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1 Introduction

Sexual communication in most moths is dependent on the female's ability to relay information regarding conspecificity, reproductive status, and location to receptive males. Research in earnest into the underpinnings of this chemical-based sexual communication originated with the seminal structure elucidation study published more than 50 years ago by Butenandt and co-workers (Butenandt et al. 1959). In that study, the authors reported the first chemical identification of an insect sex pheromone, (*E,Z*)-10,12-hexadecadien-1-ol (i.e. bombykol), extracted from 500,000 female silkworm moth (*Bombyx mori*) abdominal glands. Similar herculean efforts lead to the structural identification of sex pheromones from the cabbage looper *Trichoplusia ni* (Berger 1966) and the gypsy moth *Lymantria dispar* (Bierl et al. 1970). Since then, advances in analytical methodologies have facilitated elucidation of sex pheromones from several hundred lepidopteran species (El-Sayed 2014).

Sex pheromones are frequently de novo synthesized as multicomponent blends from acetyl-CoA (a process termed pheromonogenesis) in a specialized organ commonly referred to as the pheromone gland (PG) that is comprised of a single layer of modified epidermal cells between the eighth and ninth abdominal segments (Tillman et al. 1999; Jurenka 2003). Most moths produce Type I sex pheromones, which consist of long, straight chain hydrocarbons ($C_{10–18}$) with varying double bonds and functional modifications (alcohol, aldehyde, or acetate ester) of the carbonyl carbon (Tillman et al. 1999; Jurenka 2003, 2004; Ando et al. 2004). In contrast, Type II sex pheromones account for a small percentage (~15%) of the known lepidopteran compounds and are characterized by unmodified carbonyl carbons that consist of longer polyunsaturated hydrocarbons ($C_{17–23}$) and their epoxide derivatives (Ando et al. 2004; also see Chap. 11 volume 2). Early research on sex pheromone biosynthetic pathways clearly established that fatty acid metabolism intermediates (e.g. palmitic acid/hexadecanoic acid) provided the framework for downstream modifications. Using radiolabeled precursors, researchers were further able to elucidate specific biochemical steps to determine that pheromonogenesis, at least of the Type I pheromones, was derived from the dynamic interplay of selective β-oxidation reactions (i.e. chain-shortening), unique desaturases, and diverse reductive modifications (Bjöstad et al. 1987).

Despite years of foundational biochemical/chemical research, continued interest in the sex pheromone field has been fueled by its clear potential in integrated pest management strategies (Witzgall et al. 2008, 2010) and the ability to offer intriguing evolutionary insights (Roelofs et al. 2002; Lassance et al. 2010; Albre et al. 2013). Recent advances in genome/transcriptome sequencing, expansion of available molecular databases, and the advent of gene knockdown/knockout methodologies (e.g. RNA interference, CRISPR and TALENs) have greatly facilitated our understanding of moth pheromonogenesis at the cellular and molecular levels. This review will focus on the molecular mechanisms governing initiation and propagation

of the signal that drives moth pheromonogenesis with a final section highlighting studies that describe approaches to disrupt and/or exploit this critical pathway.

2 Regulation of Pheromonogenesis

2.1 Hormonal and Neuroendocrine Regulation

2.1.1 Hormonal Regulation

Early observations that the production and release of pheromones in some insect species coincided with female reproductive cycles lead to the hypothesis that pheromone production was hormonally regulated (Barth 1965). The two predominant hormones in insects, juvenile hormone (JH) and 20-hydroxyecdysone (20E), are now recognized as critical regulators of pheromone production in cockroaches (Schal et al. 2003), beetles (Seybold and Vanderwel 2003; Haberer et al. 2010), flies (Wicker-Thomas et al. 2009; Bilen et al. 2013), ants (Cuvillier-Hot et al. 2004; Holman 2012), and wasps (Kelstrup et al. 2014). In moths, the role of JH varies. For relatively long-lived moths, in which sex pheromone production is delayed and activities related to migration and reproduction are asynchronous (i.e. noctuid species such as the armyworm *Pseudaletia unipuncta*, the black cutworm moth *Agrotis ipsilon,* and the cotton bollworm *Heliothis armigera*), JH functions in the control of pheromone production (Cusson and McNeil 1989; Picimbon et al. 1995; Fan et al. 1999; Zhou et al. 2000). In *A. ipsilon*, JH stimulates the release of a peptidergic factor (see Sect. 2.1.2) from production sites in the brain to trigger pheromone production in 4-day old sexually mature females (Picimbon et al. 1995). In species with shorter lifespans, such as *H. armigera*, in which females initiate pheromone production at an earlier stage, JH (JH-II) primes the female PG to respond to the peptidergic factor (Fan et al. 1999). Conversely, JH has also been implicated in pheromonostasis, i.e. suppression of pheromone production after mating (Webster and Cardé 1984). Exogenous JH has been shown to suppress pheromone production in some moth species (Rafaeli and Bober 2005; Bober et al. 2010; Zhang et al. 2014b), and the male-derived sex peptide that mediates the post-mating behavioral switch in *Drosophila* has both allatotropic (triggering JH biosynthesis) and pheromonostatic effects in *H. armigera* (Fan et al. 1999, 2000; Hanin et al. 2012).

Non-JH hormonal factors from the bursa copulatrix have also been reported to be required for pheromone production in the redbanded leafroller (*Argyrotaenia velutinana*), the eastern spruce budworm (*Choristoneura fumiferana*) and the oblique banded leaf roller (*C. rosaceana*) (Fabriàs et al. 1992; Delisle et al. 1999). It has been postulated that the relative importance of the bursa copulatrix in the hormonal regulation of pheromone production may be related to the evolution of enzyme desaturation systems in specific pheromone biosynthetic pathways, as found for instance in tortricid moths (Delisle et al. 1999).

2.1.2 Neuroendocrine and Neural Regulation

Pheromonogenic control in yet other moth species has been shown to proceed by a non-hormonal mechanism, as surgical removal of the *corpora allata* (CA; site of JH synthesis) had no discernible effect on the calling behavior of female saturnid moths (Riddiford and Williams 1971) and injection of CA homogenates also failed to stimulate pheromone production in *Helicoverpa* (*Heliothis) zea* (Raina and Klun 1984). Furthermore, circadian oscillations in pheromone production and emission coinciding with specific points of the day:night cycle (Raina and Klun 1984; Hunt and Haynes 1990; Delisle and Royer 1994; Kamimura and Tatsuki 1994; Gemeno and Haynes 2000; Foster 2000; Rosén 2002; Mazor and Dunkelblum 2005; Fónagy et al. 2011; Bloch et al. 2013; see Chap. 7) and the presence of a circulating pheromonogenic factor in the hemolymph of moths during scotophase (Ichikawa 1998; Jacquin et al. 1994; Ramaswamy et al. 1995) suggested a neuroendocrine component to pheromonogenic regulation. Biochemical analyses using adult *H. zea* females revealed that the factor was a peptide hormone, subsequently purified to homogeneity (see Sect. 2.2.1) and designated Pheromone Biosynthesis Activating Neuropeptide (PBAN), that was present in the brains and subesophageal ganglion (SOG) (Raina and Klun 1984; Raina et al. 1989). Accumulating evidence has supported circadian regulated release of PBAN from the *corpora cardiaca* into the hemolymph for direct pheromonotropic activity on PGs. However, reports describing pheromonotropic activity of a PBAN-like immunoreactive factor in the ventral nerve cord (VNC) and terminal abdominal ganglion, along with impaired pheromone production after severing the VNC suggest regulation may involve a neural component as well (Marco et al. 1996; Iglesias et al. 1998; Teal et al. 1999; Rosén 2002).

Neural signals from the VNC and depletion of sperm in the spermatheca are also important post-copulatory factors that regulate post-mating inhibition of pheromone production in polyandrous moths (Delisle et al. 2000; Delisle and Simard 2002). Mated females of polyandrous (multiple matings) species usually display a refractory period to reproduction after mating, which is largely due to the transfer of male humoral factors (sperm and seminal fluid) during copulation. Some of these male factors have short-term effects, whereas others can induce long-term suppression of female receptivity, as described in both butterflies and moths (Wedell 2005).

2.2 Purification and Characterization of the Pheromone Biosynthesis Activating Neuropeptide (PBAN)

2.2.1 HPLC-Based Identification of PBAN

Determination that the moth phermonotropic factor (i.e. PBAN) was a peptide hormone present in the brains and SOG of adult *H. zea* females facilitated HPLC (high-performance liquid chromatography) purification of the 33-amino acid PBAN from

2500 *H. zea* female brain-SOG complexes (Raina et al. 1989). Neuropeptides with similar functionalities and moderate overall sequence homology were likewise purified to homogeneity and sequenced from *B. mori* (Kitamura et al. 1989, 1990) and *L. dispar* (Masler et al. 1994). Consistent with its presumed role as the cue driving circadian oscillations in pheromone production, PBAN levels in both the brain and hemolymph fluctuate in accordance with photoperiod (Rafaeli et al. 1991, 1993; Rafaeli 1994; Ramaswamy et al. 1995; Iglesias et al. 2002; Nagalakshmi et al. 2007; Závodská et al. 2009). All PBANs have a conserved FxPRL-NH_2 (Phe-Xxx-Pro-Arg-Leu-amide) C-terminal pentapeptide motif that is critical for pheromonotropic activity (Raina and Kempe 1990; Kitamura et al. 1989). In addition, these pheromonotropic peptides exhibited species cross-reactivity as well as functional cross-reactivity with locust myotropins and tachykinins (Kuniyoshi et al. 1992; Fónagy et al. 1992a, b; Nachman et al. 1993a, b), suggesting that the cognate FxPRL-NH_2 peptide receptors were also similar.

2.2.2 Structure-Function Analysis of PBAN

Initial structure-function analyses of PBAN examined the pheromonotropic efficacy of peptide fragments generated either as a series of N-terminally truncated synthetic peptides (Raina and Kempe 1990) or endoproteinase Glu-C fragments (Kitamura et al. 1989). In both studies, the minimal sequence needed to stimulate pheromone production consisted of the C-terminal pentapeptide core (i.e. FxPRL). Comparison of amidated, hydroxylated, and methyl ester versions of the pentapeptide revealed the critical importance of the C-terminal amide (Kitamura et al. 1989; Kuniyoshi et al. 1992; Nagasawa et al. 1994). Sequential amino acid substitution of the core pentapeptide motif in *B. mori* (FSPRL-NH_2) revealed that Phe and Ser could be replaced with similar residues with little disruption of pheromonotropic activity, whereas Pro, Arg, and Leu could not (Kuniyoshi et al. 1991). Comparison of the pheromonotropic efficacies of FxPRL-NH_2 peptides from diverse species provided further insights into the structure-function relationships and suggested that the variable "x" position had greater pheromonotropic properties if occupied by Thr compared to Val, Ser, or Gly (Abernathy et al. 1995). More recent structure-function analyses revealed that the positively charged basic Arg (R; two positions from the C terminus) is the most critical residue within the hexapeptide motif (Kim et al. 2008; Kawai et al. 2012). It is followed in importance by the branched chain Leu, aromatic Phe, and then to a lesser extent by the other residues (Kim et al. 2008).

To provide greater insights into the role the individual residues in the C-terminal pentapeptide motif might play in receptor activation, Nachman and co-workers used nuclear magnetic resonance (NMR) spectroscopy, circular dichroism, and molecular dynamics simulations to determine that a cyclic analog of the pentapeptide adopts a C-terminal β turn in solution (Nachman et al. 1991). The analog, which introduced significant conformation constraints and increased the overall rigidity of the pentapeptide, retained biological activity, indicating that this conformation is crucial for receptor activation. Molecular simulations using the linear pentapeptide

active core suggested the conformation was not specific to the cyclization process. Subsequent NMR analyses of a hexapeptide (TFSPRL-NH_2) analog and the full-length *H. zea* PBAN confirmed that the peptide assumes a C-terminal type I′ β turn in solution (Wang et al. 1994; Clark and Prestwich 1996). A more recent NMR study of an 18-amino acid pheromonotropin from *Pseudaletia separata* characterized by a C-terminal FxPRL-NH_2 revealed an extended β sheet structure devoid of the previously identified β turn (Bhattacharya et al. 2015). However, that study was performed in water as opposed to a more polar solvent (e.g. trifluoroethanol/water or dimethyl sulfoxide/water) that would presumably more accurately mimic the lipid bilayer environment in which the cell surface receptors are embedded.

2.3 Molecular-Based identification of PBAN

2.3.1 PBAN Transcripts

Following purification of the respective PBANs, cloning methods employing sequence information provided by the isolated peptides facilitated molecular elucidation of the *B. mori* and *H. zea* PBAN gene products (Davis et al. 1992; Kawano et al. 1992; Sato et al. 1993; Ma et al. 1994). In both instances, post-translational proteolytic processing of the encoded open reading frames was predicted to yield the respective PBANs and four additional peptides with C-terminal FxPRL-NH_2 motifs identified as diapause hormone (DH) and α, β, and γ subesophageal neuropeptides (i.e. SGNPs). Among the four additional peptides, DH had previously been isolated to homogeneity and shown to function in embryonic diapause (Imai et al. 1991). Synthetic α, β, and γ SGNPs were reported to have pheromonotropic activity in *H. zea* (Ma et al. 1994), but in *B. mori* the α and γ SGNPs were less effective than PBAN (β SGNP was comparable) at stimulating pheromone production and all three were less potent than DH in diapause induction (Sato et al. 1993). Later studies using PBANR receptors heterologously expressed in *Xenopus* oocytes, however, reported that the three SGNPs were more potent than PBAN in generating chloride currents (Watanabe et al. 2007).

Organization of the FxPRL-NH_2 open reading frames is conserved in both the *B. mori* and *H. zea* transcripts with the DH sequence downstream of the signal peptide followed by the α and β SGNPs, PBAN, and then γ SGNP. Since initial cloning, PBAN-encoding cDNAs with similar sequence architecture have been published for 22 lepidopterans with additional sequences deposited in GenBank or the Transcriptome Shotgun Assembly (TSA) sequence databases (Table 8.1) with most of the peptides composed of 33 residues (Fig. 8.1). Outliers include the *Ascotis selenaria cretacea* (Japanese giant looper) PBAN, which is 27 amino acids, and the 37 amino acid *Omphisa fuscidentalis* PBAN. A second 37 amino acid PBAN recently identified in *Ostrinia nubilalis* suggests close conservation of PBAN gene architecture between the closely related crambid subfamilies Pyraustinae and Spilomelinae (Fodor et al. 2017). The *A. s. cretacea* PBAN transcript is also unique

Table 8.1 Accession numbers for PBAN and PBANR sequences identified in lepidopteran species

PBAN		PBANR	
Species	GenBank protein accession no.	Species	GenBank protein accession no.
Published sequences			
Adoxophyes sp.	AAK72980	*Agrotis segetum*	AID66638
Agrotis ipsilon	CAA08774/O76818	*Bombyx mori*	AEX31546, AEX15646, AEX15643/BAD44726, AEX15640
Antheraea pernyi	AAR17699	*Helicoverpa armigera*	AEX31547, AEX15647/AAW47417, AEX15644, AEX15641
Ascotis selenaria cretacea	BAF64458	*Helicoverpa zea*	AAP93921, AEO17028, AFP19101
Bombyx mandarina	AAM88285	*Heliothis peltigera*	AEQ33641
Bombyx mori	BAA05954/AAB24327	*Heliothis virescens*	ABU93812, ABU93813, ABV58013
Chlumetia transversa	AIY72749	*Mamestra brassicae*	ARO85771-ARO85773
Clostera anastomosis	ABR04093	*Ostrinia nubilalis*	AGL12066-AGL12068
Helicoverpa armigera	AAM43840/AAL05596/AAQ82626	*Plutella xylostella*	AAY34744/AEP25401
Helicoverpa assulta	AAC64293	*Pseudaletia separata*	AEX31548, AEX15648, AEX15645, AEX15642
Helicoverpa zea	P11159/AAA20661	*Spodoptera exigua*	ABY62317
Heliothis virescens	AAO20095	*Spodoptera littoralis*	ABD52277
Holcocerus hippophaecolus	n/a[a]		
Mamestra brassicae	AAC02094		
Manduca sexta	AAO18192		
Maruca vitrata	AGI96545		
Omphisa fuscidentalis	AFP87384		
Ostrinia nubilalis	AOY34014		
Plutella xylostella	AAX99220		
Samia cynthia ricini	AAP41132		

(continued)

Table 8.1 (continued)

PBAN		PBANR	
Species	GenBank protein accession no.	Species	GenBank protein accession no.
Spodoptera exigua	AAT64424/AAR87744		
Spodoptera littoralis	AAK84160		
Spodoptera litura	AJT60314		
Unpublished sequences (GenBank annotations only)			
Chilo suppressalis	ALM30314	*Chilo suppressalis*	ALM88337-ALM88338
Omphisa fuscidentalis	AFP87384	*Manduca sexta*	ACQ90219-ACQ90222
Orgyia thyellina	BAE94185	*Spodoptera litura*	AJW32184
Ostrinia furnacalis	BAQ21230		
Genome Annotations			
Amyelois transitella	XP_013189838	*Amyelois transitella*	XP_013187133
Danaus plexippus	EHJ67284	*Papilio machaon*	XP_014362489,XP_014362488,XP_014362487
Papilio machaon	XP_014371142	*Papilio polytes*	XP_013142894,XP_013142893,XP_013142892
Papilio polytes	XP_013144402	*Papilio xuthus*	XP_013176026, XP_013176019, XP_013176012
Papilio xuthus	XP_013168299/XP_013163175/ XP_013168300		
Transcriptome Shotgun Assembliesb			
Athetis lepigone	GARB01004345	*Actias selene*	GBZL01006651
Biston suppressaria	GCJP01035652	*Antheraea yamamai*	GBZJ01027120
Chilo suppressalis	GAJS01037377	*Athetis lepigone*	GARB01028884
Dyseriocrania subpurpurella	GASY02017090	*Biston suppressaria*	GCJP01052341
Nemophora degeerella	GATC02010886	*Cadra cautella*	GBXH01027379
Papilio zelicaon	JP623453	*Helicoverpa assulta*	GBTA01046701/GBTA01046700

Polyommatus icarus	GAST02017042	*Nemophora degeerella*	GATC02017805
Spodoptera frugiperda	GESP01042864.1	*Ostrinia furnacalis*	GAQJ01060384
Triodia sylvina	GAVB02014270	*Parides eurimedes*	GAXH02029056
Yponomeuta evonymellus	GASG02034409	*Polyommatus icarus*	GAST02014754
		Spodoptera frugiperda	GESP01096852
		Yponomeuta evonymellus	GASG02024048

[a]See Li J, Zhou J, Sun R, et al (2013) Arch Insect Biochem Physiol 82:183–195. doi: 10.1002/arch.21084
[b]tBLASTn against TSA archive (08/28/2016) using *B. mori* PBAN (AAB24327) with *e* value $<1e^{-05}$ or PBANR (BAD44726) *e* value $<1e^{-60}$

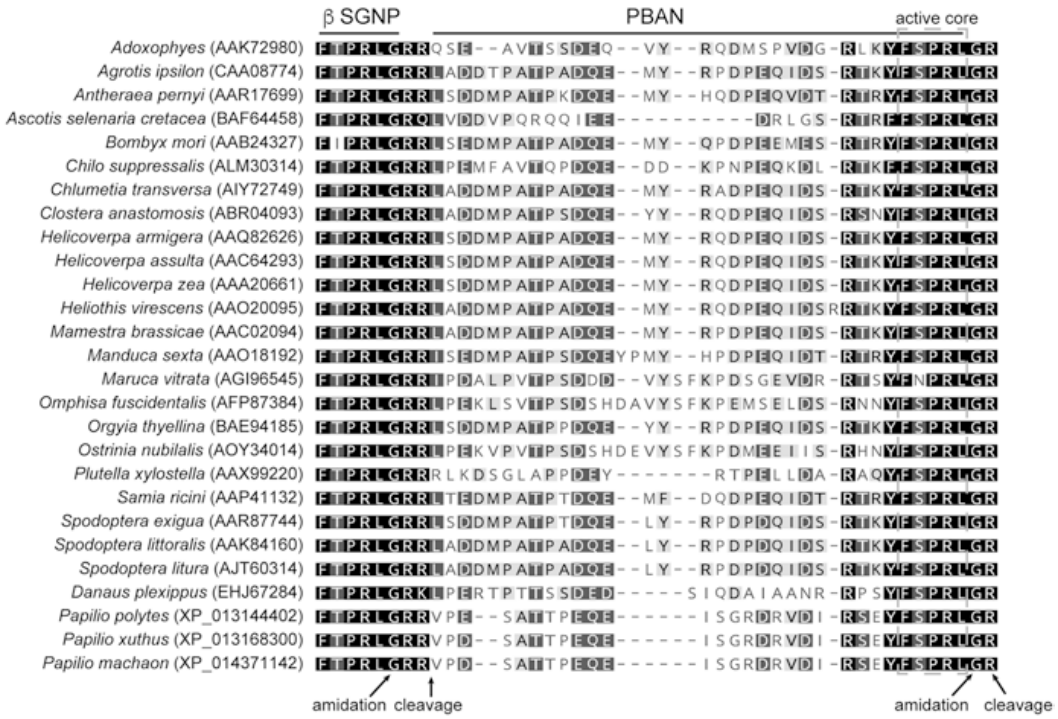

Fig. 8.1 Multiple sequence alignment of PBAN coding sequences from diverse lepidopteran species. The alignment was made using MUSCLE implemented in Geneious 7. Sequences correspond to the portion of the DH/PBAN transcript from the FxPRL portion of the β SGNP through the predicted proteolytic cleavage site at the C terminus of the PBAN sequence. Predicted cleavage and amidation sites are indicated. The essential FxPRL active core of PBAN is indicated by the dashed grey lines. Genome-based butterfly sequences are clustered at the bottom of the alignment. Protein accession numbers are indicated in parentheses

in that it generates a fused β SGNP/PBAN with a double FxPRL motif (Kawai et al. 2007). Alignment of multiple lepidopteran PBAN sequences revealed that the variable position in the FxPRL-NH_2 motif reported to have an effect on pheromonotropic activity (Abernathy et al. 1995) is a conserved Ser (Fig. 8.1). The exception is *Maruca vitrata* (legume pod borer), which has Asn, an uncharged polar residue with a bulkier sidechain than Ser (Chang and Ramasamy 2014). Furthermore, all of the published PBAN cDNAs to date contain a dibasic KK motif upstream of the α SGNP sequence. While KK cleavage has been reported to be infrequent (Veenstra 2000), proteolytic processing of the PBAN prepropeptides was confirmed via HPLC-based fractionation of *B. mori* SOGs (Sato et al. 1993) and MALDI (matrix-assisted laser desorption/ionization) mass spectrometry of individual *H. zea* SOG neuronal clusters (Ma et al. 2000).

The presence of PBAN sequence (and/or prepropeptide) variants was initially described in *B. mori* following HPLC-based purification of two peptides (PBAN-I and PBAN-II) with pheromonotropic activity that differed from one another by a single N-terminal Arg residue (Kitamura et al. 1989, 1990). Since then potential sequence variants have been deposited with the NCBI database for a number of species including *B. mori* (three point mutations between AAB24327 and

BAA05954-K109 N, M139I, and E146V), *Spodoptera litura* (one point mutation between AJT60314 and AKT95050-E53G), and *Helicoverpa armigera* (three point mutations between AAL05596 and AAM43840-deletion of N3, insertion of G before G30, and M179I).

At this point, it is uncertain if these variants represent population differences, are differentially expressed variants, or are merely the result of sequence errors introduced during cloning. However, differentially expressed PBAN preropeptide transcript variants have been reported in the sand fly *Phlebotomus papatasi* (Choi et al. 2015) and are suggested based on a band doublet observed on an RT-PCR gel of fire ant thoraces (Choi et al. 2011). More recently, transcripts that vary in the length and composition of their 3′UTRs (untranslated regions) have been identified in *O. nubilalis* (Fodor et al. 2017).

2.3.2 PBAN Gene Structure

The lepidopteran PBAN genomic structure is conserved with PBAN genes in *B. mori* (Xu et al. 1995), *H. armigera* (Zhang et al. 2005), *M. vitrata* (Chang and Ramasamy 2014), and *Clostera anastomosis* (Jing et al. 2007) encompassing six exons with identical exon coding (Fig. 8.2). Exon one encodes the signal peptide and a portion of DH, exon two the remaining portion of DH, exon three an uncharacterized peptidergic sequence, exon four the α and β SGNPs and a portion of PBAN, and exon five the remaining portion of PBAN and γ SGNP. The stop codon is located in exon six. Splicing of all four genes follows the GT-AG rule and utilizes 0, 2, 1, 2, 1 phasing; however, despite the similarities, the overall sizes of the genes differ with varying intron lengths (Fig. 8.2). The *O. nubilalis* PBAN was recently reported to have the same genomic structure (Fodor et al. 2017).

Limited promoter analyses, which focused on elucidating how DH expression was regulated in relation to embryonic diapause as opposed to pheromonogenesis, identified potential differences in transcription between the *B. mori* and *H. armigera* genes. POU-M2, a eukaryotic transcription factor with a bipartite DNA binding domain implicated in neuroendocrine function, activated expression from the *B. mori* PBAN promoter *in vitro* but failed to do so with a conserved region of the *H. armigera* promoter (Zhang et al. 2004a, 2005). In contrast, an E-box element (CAGCTG) present in the *H. armigera* promoter was reported to be critical for transcriptional activation (Hong et al. 2006), which was dependent on co-ordinate interactions with upstream activating and inhibitory regions. Taken together, the findings suggest that the two species utilize variations in transcriptional regulation to drive the respective differences in diapause programs. Additionally, an ecdysone response element was identified in the promoter region of the *B. mori* PBAN gene (Xu et al. 1995). While ecdysteroids have not been associated with diapause control, they are critical regulators of lepidopteran reproduction (Van Wielendaele et al. 2013; De Loof et al. 2016). Consequently, the response element may link PBAN transcription with reproductive competence; however, the role it has in pheromonogenesis remains to be revealed.

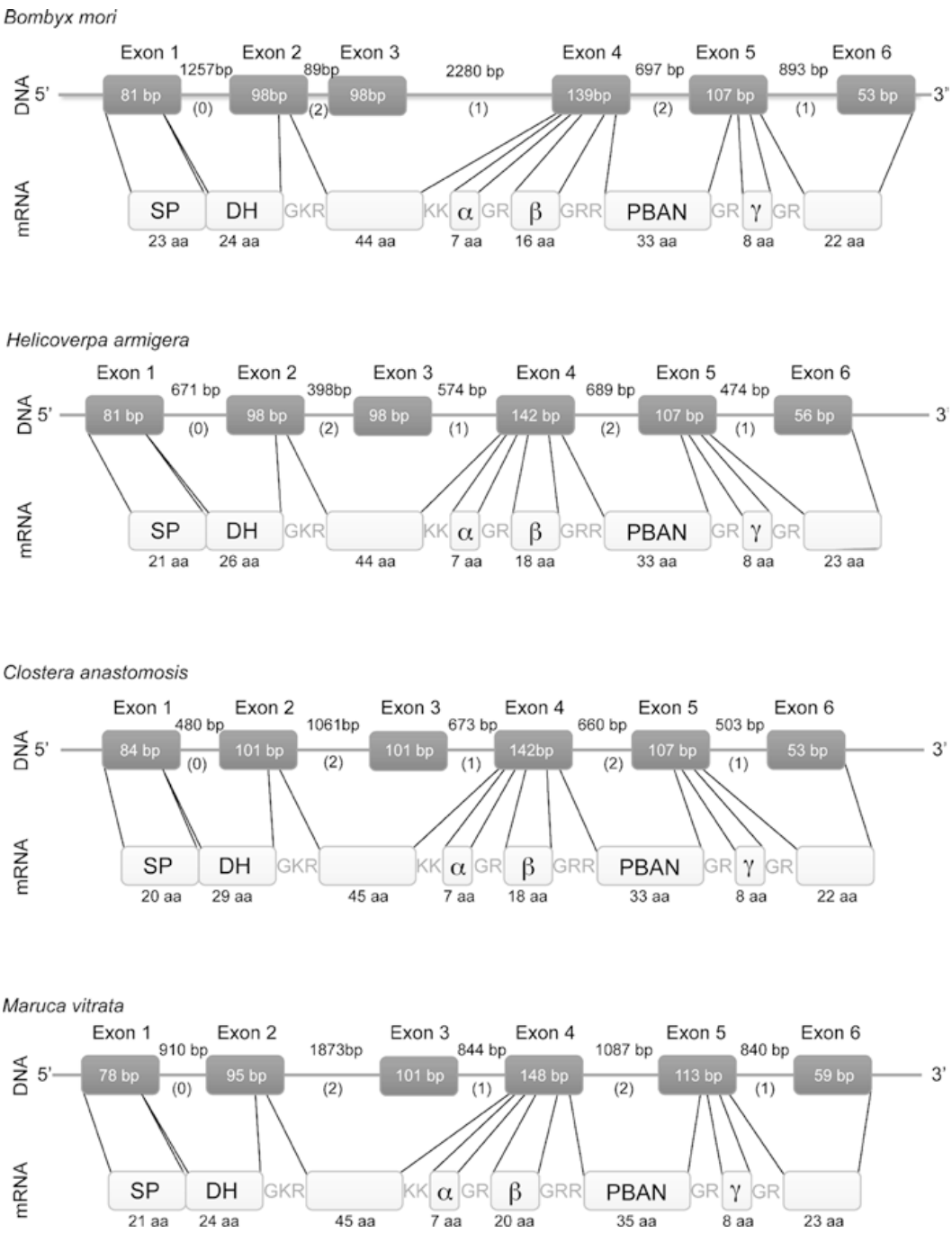

Fig. 8.2 Genomic organization of the DH-PBAN gene in four moth species. Schematic comparison of genomic DNA and the translated peptides for the DH-PBAN gene in *Bombyx mori* (Xu et al. 1995), *Helicoverpa zea* (Zhang et al. 2005), *Clostera anastomosis* (Jing et al. 2007), and *Maruca vitrata* (Chang and Ramasamy 2014). Darker shaded boxes indicate exons, whereas lighter shaded boxes indicate the encoded peptides. Horizontal solid lines represent introns with the corresponding intron phase in parentheses. GKR, KK, GR, and GRR indicate probable endoproteolytic cleavage sites. SP - signal peptide; DH-diapause hormone; α-α SGNP; β-β SGNP; PBAN - pheromone biosynthesis activating neuropeptide; γ-γ SGNP. Note, while the sizes of exons and introns are indicated, the models are not drawn to scale

2.4 Other FxPRL-NH_2 Peptides

The critical C-terminal pentapeptide is now recognized as a defining feature of the PBAN/pyrokinin (FxPRL) family of pleiotropic neuropeptides present throughout Insecta and includes pyrokinins, PBANs, myotropins, DH, and the α, β, and γ SGNPs (Predel and Nachman 2006; Jurenka and Nusawardani 2011; Altstein et al. 2013; Jurenka 2015; Yaginuma and Niimi 2015). In addition to the pheromonotropic effects in moths, FxPRL-NH_2 peptides also regulate the induction of cuticular melanization in moth larvae (Matsumoto et al. 1992; Altstein et al. 1996), the induction of embryonic diapause and seasonal polyphenism in moths (Imai et al. 1991; Uehara et al. 2011), the termination of pupal diapause in heliothine moths (Xu and Denlinger 2003; Zhang et al. 2004b, c; Zhao et al. 2004), prothoracic gland ecdysteroidogenesis (Zhang et al. 2004c; Watanabe et al. 2007), visceral muscle contraction in cockroaches (Holman et al. 1986; Nachman et al. 1986; Predel et al. 2001), acceleration of puparium formation in flies (Zdárek and Nachman 1997; Zdárek et al. 1998, 2002, 2004), production of fatty acid components in male *H. armigera* hair-pencil aedeagus complexes (Bober and Rafaeli 2010), and the biosynthesis of trail pheromones in *Solenopsis invicta* (Choi and Vander Meer 2012). This multifunctionality is similar to the structural variation described for the chemosensory protein (CSP) family of multi-function transporters, which are widely expressed in diverse tissues including the PG (see Chaps. 6, 9, and 10, volume 2; Xuan et al. 2014, 2016; Picimbon 2017).

PBAN control of pheromonogenesis, however, is not ubiquitous throughout the moths (Tang et al. 1989; Subchev and Jurenka 2001; Fujii et al. 2010) nor is it specific to moths that produce Type I pheromone components (albeit our knowledge of this system is more complete) as it has been reported to regulate production of Type II pheromones in the giant looper *A. s. cretacea* (Wei et al. 2004; Fujii et al. 2007). Furthermore, some Lepidopteran species such as *T. ni* do not exhibit diel periodicity in pheromone production (Hunt and Haynes 1990; also see references in Rafaeli and Jurenka 2003; Altstein 2004a), and as such would be expected to have little need for PBAN-mediated regulation. However, *T. ni* brain extracts were found to have pheromonotropic activities in other moth species (Tang et al. 1989). Since then it has become apparent that PBAN is a pleiotropic regulator of diverse activities (see above). Indeed, the elucidated primary structure of the HPLC-purified *B. mori* peptide responsible for larval cuticular melanization (i.e. melanization and reddish coloration hormone) was identical to the PBAN sequence (Matsumoto et al. 1990).

2.5 *Identification of the PBAN Receptor (PBANR)*

2.5.1 PBANR: Early Studies

The involvement of a cell surface receptor that mediates the pheromonotropic effects of PBAN was demonstrated early on following direct stimulation of dissected PGs by PBAN (Soroker and Rafaeli 1989; Jurenka et al. 1991b; Fónagy et al. 1992a, c). Pharmacological profiling with NaF (sodium fluoride), a potent G protein activator that had pheromonotropic effects (Rafaeli and Gileadi 1996a, b) further pointed to the involvement of a G protein-coupled receptor (GPCR). The photoaffinity labeling of a ~50 kDa membrane protein in *H. armigera* PG cells with a biotinylated PBAN analog provided incontrovertible evidence of a PG-derived cell surface protein (Rafaeli and Gileadi 1999; Rafaeli et al. 2003, 2007). However, molecular identification of the moth PBAN receptor (PBANR) ultimately depended on publication of the *Drosophila melanogaster* genome (Adams et al. 2000).

2.5.2 Homology-Based Cloning of PBANR

Sequence homologies between mammalian receptors and putative GPCRs in the *Drosophila* genome led researchers to propose that co-evolution of receptors and their ligands would yield closely aligned receptor families (Hewes and Taghert 2001). Based on this hypothesis, similarities in the active core of FxPRL-NH_2 peptides and neuromedin U (FRPRN-NH_2) suggested that the respective receptors are evolutionarily related. Functional analyses demonstrated that three *Drosophila* GPCRs (CG8784, CG8795, and CG9918) that clustered in phylogenetic analyses with the neuromedin U receptor (NmUR) clade were activated to varying degrees by FxPRL-NH_2 peptides (Park et al. 2002). A subsequent study reported pheromonotropic effects of mammalian NmU in *H. zea*, which further bolstered the receptor co-evolution hypothesis and showed that homology-based methods could be used to clone receptors from the NmUR clade (Choi et al. 2003). The *H. zea* GPCR identified in that study was amplified from PG cDNAs and, when heterologously expressed in cultured Sf9 cells, dose-dependently triggered an influx of extracellular Ca^{2+} in response to synthetic *H. zea* PBAN. This was interpreted as evidence that the authors had identified the first PBANR (i.e. HelzePBANR). Using a similar approach, the *B. mori* PBANR (BommoPBANR) was likewise cloned from PG cDNAs. BommoPBANR mobilized extracellular Ca^{2+} in response to PBAN stimulation, had significant sequence similarity with NmUR homologs, and was up-regulated on the day preceding adult eclosion (Hull et al. 2004), a time period that coincides with *B. mori* pheromonogenesis (Matsumoto et al. 2007, 2010).

2.5.3 The Complexity of PBANR

Identification of PBANR Variants

Perplexingly, the ~50 kDa protein labeled with the biotinylated PBAN analog in the intersegmental membranes that comprise the *H. armigera* PG (Rafaeli and Gileadi 1999; Rafaeli et al. 2003, 2007) was closer in size to BommoPBANR (45.9 kDa) than the smaller HelzePBANR (38.6 kDa). Despite presumably mediating similar biological responses and significant sequence identity through the seventh transmembrane domain (TM7), BommoPBANR was differentiated by the presence of a 67-aa C-terminal extension critical for ligand-induced internalization (Hull et al. 2004, 2005), an endocytotic mechanism associated with GPCR feedback regulation and desensitization (Moore et al. 2007; Marchese et al. 2008). Further confounding the issue, PBANRs subsequently identified in *H. armigera* (Rafaeli et al. 2007), *S. littoralis* (Zheng et al. 2007), *Spodoptera exigua* (Cheng et al. 2010), and *Plutella xylostella* (Lee et al. 2011) also lacked the C-terminal extension, suggesting feedback regulation of these receptors differed from BommoPBANR. The prevalence of the "short" PBANRs raised questions concerning the evolutionary significance of the BommoPBANR extension. Initially, comparisons were made with type I gonadotropin-releasing hormone receptors in which non-mammalian receptors have a C-terminal tail and undergo rapid ligand-induced internalization, whereas mammalian receptors lack the extended C terminus and have significantly different internalization kinetics (Pawson et al. 1998; McArdle et al. 2002). The potential biological significance of the "short" and "long" PBANRs also led to speculation that the varied C-terminal lengths reflected differences in the importance of the second messenger 3′,5′-cyclic adenosine monophosphate (cAMP) in the respective species. The identification of three PBANR variants concomitantly expressed in *Helicoverpa virescens* (referred to as HelviPBANR A-C) with a conserved N-terminal sequence, but with differing C-terminal lengths (Kim et al. 2008), further underscored the complexity of the PBAN signaling system. Similar to BommoPBANR, the HelviPBANR-C variant has an extended C terminus and contains a defined internalization motif (see 2.7.7), whereas the C-terminal end of the HelviPBANR-A variant resembles HelzePBANR. Moreover, HelviPBANR-C was preferentially amplified from PGs and generated robust Ca^{2+} mobilization responses following stimulation with *H. zea* PBAN. In contrast, the other two variants were amplified from larval tissues and failed to respond to the concentration of the synthetic PBAN assayed (Kim et al. 2008). These results initiated a re-evaluation of the species-specific "short" and "long" PBANR paradigm.

Using modified cloning methods, multiple PBANR variants (PBANR-As, -A, -B, and -C) were amplified from PGs of *B. mori*, *H. zea*, *H. armigera*, and *P. separata* (also referred to as *Mythimna separata*) that differed only in the length of their respective C-terminal ends (Fig. 8.3a). Similar to *H. virescens*, the most abundant PG transcripts were the "long" PBANR-C variants (Fig. 8.3b), all of which underwent ligand-induced internalization (Lee et al. 2012a). In contrast, the "short" PBANR-A variants were less abundant, mobilized extracellular Ca^{2+} poorly in

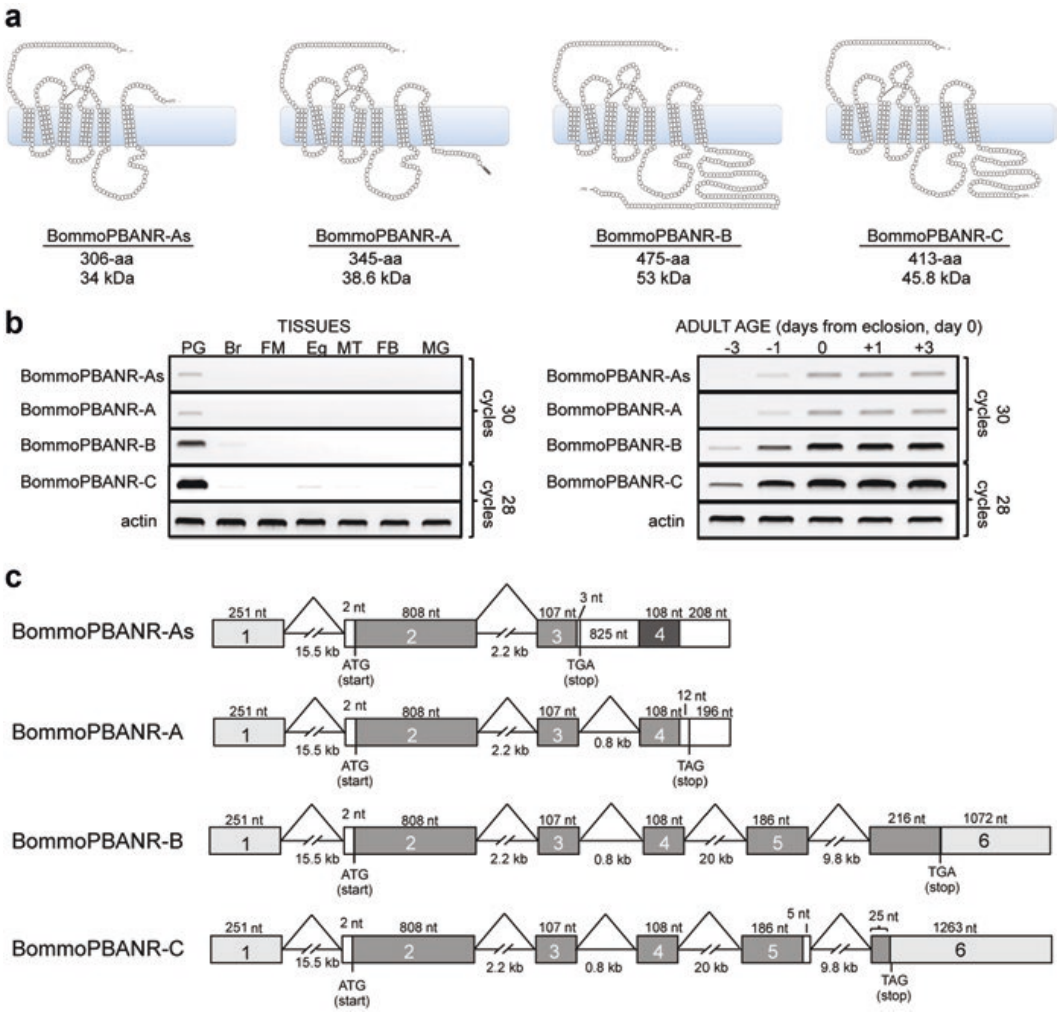

Fig. 8.3 Identification of multiple PBANR variants in *Bombyx mori* pheromone gland. (**a**) Schematic diagram depicting the sizes and structures of the various BommoPBANR variants cloned. (**b**) RT-PCR based expression profile of BommoPBANR variants in various tissues and at varying developmental time points relative to adult emergence (day 0). Abbreviations: *PG* pheromone gland, *Br* brain, *FM* flight muscle, *Eg* unfertilized egg, *MT* Malpighian tubule, *FB* fat body, *MG* midgut. This research was originally published in Frontiers of Endocrinology. Lee et al. (2012a). (**c**) Genomic organization and alternative splicing of the *Bombyx mori* PBANR gene. The four BommoPBANR variants (As, **A**, **B**, and **C**) are depicted. Light grey shading corresponds to untranslated exons, medium grey to translated exons, and dark grey to a non-translated exon that is unique to the As variant. Non-shaded boxes indicate non-spliced intronic sequences. Initiation (ATG) and stop sites (TGA or TAG) are indicated by their respective codons. (Figure adapted from Lee et al. 2012a)

response to a range of PBAN concentrations, and exhibited different internalization kinetics (Lee et al. 2012a, b). Previous preferential amplification of the shorter variants (Choi et al. 2003; Rafaeli et al. 2007; Cheng et al. 2010; Lee et al. 2011) was attributed to the high GC content (55–80%) of the extended C-terminal ends (Lee et al. 2012a), which can reduce PCR amplification efficiencies by serving as pause

or termination sites (McDowell et al. 1998). Thus it is now apparent that the ~50 kDa protein labeled with the biotinylated PBAN analog (Rafaeli and Gileadi 1999; Rafaeli et al. 2003, 2007) was most likely the HelarPBANR-C variant (51.1 kDa) rather than a glycosylated HelarPBANR-A variant (38.7 kDa) as first proposed.

PBANR Variants Arise from Alternative Splicing

Alternative splicing has been extensively documented for GPCRs (Minneman 2001; Markovic and Challiss 2009) and is one of the principal means by which organisms generate functional protein diversity in a temporal- and/or tissue-dependent manner. The modular aspect of the PBANR variants (i.e. variation specific to the C terminus) is consistent with alternative splicing. The availability of the *B. mori* genome (Mita et al. 2004; Duan et al. 2010) allowed further exploration of that hypothesis. BommoPBANR localizes to a >50 kb segment of chromosome 12 and encompasses six exons and five introns (Fig. 8.3c). The N terminus through the last transmembrane domain (i.e. TM7) are encoded on exons 2–4, the C terminus on exons 5–6, and the 5′ untranslated region on exon 1. The introduction of premature stop codons following retention of introns 3 or 4 yields the BommoPBANR-As and BommoPBANR-A variants, respectively. BommoPBANR-C arises from a five-nucleotide frame shift insertion at the 3′ end of exon 5 that changes codons for the remaining ten amino acids (residues 404–413) and introduces a stop codon that generates a 67 amino acid C terminus. In contrast, BommoPBANR-B is generated from conventional splicing of exons 2–6 (Lee et al. 2012a). As more lepidopteran genomes become available, it will be interesting to see if the splicing mechanisms that generate the BommoPBANR variants are conserved in other species and what cellular/transcriptional factors trigger those splicing events.

PBANR Variants: Fine-Tuning the PBAN Signal?

To date, PBANRs have been reported or annotated in 15 species (Table 8.1) with multiple variants present in *O. nubilalis* (Nusawardani et al. 2013), *Manduca sexta* (FJ240221-FJ240224), *Chilo suppressalis* (KT031039-KT031040), *Mamestra brassicae* (Fodor et al. 2018), and based on genomic sequencing data, three *Papilio* species.

While the biological significance of concomitant expression of multiple PBANRs in PGs remains to be determined, one possibility is that they provide a mechanism for fine-tuning cellular responsiveness to the respective PBAN signals. In one model, nominally non-responsive PBANR-A receptors expressed at the cell surface could potentially function as ligand sinks that compete with PBANR-C for ligand binding. The net result would be less bioactive peptide available to trigger the cellular response thus decreasing overall sensitivity. In a second model, heterodimerization of the shorter variants with the longer variants could impede trafficking to

the cell surface, thereby decreasing the pool of available receptors for ligand binding, which would likewise decrease overall cellular sensitivity. When co-expressed in cultured cells with their predominant full-length receptor forms, truncated variants of some mammalian receptors have been reported to exert dominant negative effects on signaling (Seck et al. 2005; Zmijewski and Slominski 2009; Chow et al. 2012). Alternatively, because many receptor variants exhibit distinct spatial and temporal expression profiles as well as altered ligand binding, atypical feedback regulation, and differential activation of downstream effector pathways (Markovic and Challiss 2009), the multiple PBANR transcripts may reflect a spatio-temporal dependence of functionality. This hypothesis is especially attractive given the pleiotropic complexity of PBAN, the multiplicity of reports detailing PBANR activation by multiple FxPRL-NH_2 peptides (Choi et al. 2003; Watanabe et al. 2007; Kim et al. 2008; Hariton-Shalev et al. 2013; Shalev and Altstein 2015), and the varied expression profile of PBANR transcripts, which have been amplified from diverse tissues including the PG, brain, SOG, ventral nerve cord, thoracic ganglion, ovary, and male abdominal tip (Rafaeli et al. 2007; Watanabe et al. 2007; Bober and Rafaeli 2010; Cheng et al. 2010). Indeed, PBANR expression in larval tissues (Zheng et al. 2007; Kim et al. 2008) suggested possible roles in melanization and/or pupal diapause. Recent studies seem to support this hypothesis with larval-derived and PG-derived PBANRs differing markedly in their three-dimensional conformations, regions/degrees of electrostatic potential, and ligand binding properties (Hariton-Shalev et al. 2013; Shalev and Altstein 2015). While suggestive, these findings require further validation using alternative expression systems, the inclusion of more PBANRs, and the use of various potential endogenous ligands.

2.6 Other FxPRL-NH_2 Receptors

Although significant progress has been made in molecular characterization of PBANRs, the presence of transcripts in diverse tissues, pleiotropic activation (i.e. DH, PBAN, and SGNPs), and the concomitant expression of multiple variants have collectively raised questions regarding the spatio-temporal interactions between the receptor and the FxPRL-NH_2 peptides that regulate pheromonogenesis. These questions were both clarified (and further obscured) following identification of the *B. mori* DH receptor (BommoDHR) (Homma et al. 2006). DH is one of the five FxPRL-NH_2 peptides encoded on the PBAN prepropeptide gene and functions in induction of embryonic diapause and seasonal polyphenism (Imai et al. 1991; Uehara et al. 2011), the termination of pupal diapause in heliothine moths (Xu and Denlinger 2003; Zhang et al. 2004b; Zhao et al. 2004), and prothoracic gland ecdysteroidogenesis (Zhang et al. 2004c; Watanabe et al. 2007). Although BommoDHR was cloned from developing ovaries using a homology-based approach similar to that used for the PBANRs, sequence identity between BommoDHR and the BommoPBANR variants is only ~40% (Homma et al. 2006). DHRs have since been either cloned or identified based on sequence homology from a number of

lepidopterans (Jurenka and Nusawardani 2011). The two receptor types, along with homologs in other insect orders referred to as pyrokinin 1 receptor (PKR1; DHR-like) and pyrokinin 2 receptor (PKR2; PBANR-like), are phylogenetically distinct (Jurenka and Nusawardani 2011; Nusawardani et al. 2013; Jiang et al. 2014). Despite these differences, the activities of DH and PBAN on HelzePBANR and BommoPBANR were reported to be comparable (Choi et al. 2003, 2007; Watanabe et al. 2007). Conversely, PBAN had >20-fold lower activity on BommoDHR (Homma et al. 2006) and no activity on OstnuDHR (Nusawardani et al. 2013), suggesting that greater ligand discrimination occurs with DHR than PBANR. However, functional analyses performed by other groups using different expression systems and assays, came to different conclusions as DH had 15-fold lower activity than PBAN on HelviPBANR-C (Kim et al. 2008) and PBAN activity on HelzeDHR was virtually indistinguishable from DH (Jiang et al. 2014). While these discrepancies likely reflect methodological variances and/or complications associated with heterologous expression (Zhang et al. 2014a), in vitro differences in the efficacy of the two peptides (Stern et al. 2007; Watanabe et al. 2007; Hariton-Shalev et al. 2013; Shalev and Altstein 2015) support regulation of distinct functionalities by the respective ligand-receptor pairs. However, the reduction in pheromonogenesis observed in response to RNA interference (RNAi)-mediated knockdown of PBANRs in *B. mori* (Ohnishi et al. 2006), *P. xylostella* (Lee et al. 2011), and male *H. armigera* (Bober and Rafaeli 2010) have provided unequivocal demonstration of PBANR involvement in mediating the biological effects of PBAN. In those studies, pheromonogenesis was only partially inhibited (~50% reduction) not abolished, suggesting limited penetrance of the dsRNA into the PG cells or that receptor levels, while reduced, were still sufficient to propagate the pheromonogenic signal. Alternatively, those findings may indicate that a full pheromonogenic effect depends on additional endocrine signals and/or other FxPRL-NH_2 receptor/ligand pairs. Despite increasing the complexity of our model for pheromone regulation, the latter hypothesis is attractive as transcripts for both PBANR and DHR have been amplified from PG cDNAs (Watanabe et al. 2007; Nusawardani et al. 2013).

2.7 Structure-Function Analysis of PBANR

2.7.1 Elucidating GPCR Structural Requirements Critical to Ligand Binding and Activation

Targeted disruption of insect neuropeptide signaling, which modulates virtually all aspects of insect biology, physiology and behavior, has been proposed as a novel pest control strategy with great potential for development by the agro-chemical industry (Altstein and Nässel 2010; Audsley and Down 2015). Successful exploitation of this strategy, however, requires a comprehensive understanding of the molecular mechanisms underlying ligand binding and receptor activation. Efforts to determine the atomic structures of GPCRs by standard NMR and X-ray

crystallography methods were initially hampered by the necessity of a lipid bilayer suspension. Consequently, researchers turned to in silico methods using structurally related templates and/or structure-function analyses of GPCR mutants to gain insights into GPCR functionality. Chimeric receptors that incorporate domains from distant, but related GPCRs have also provided insights into the molecular determinants that govern ligand-receptor interactions (Yin et al. 2004) and revealed roles for the N terminus and extracellular loops (ECL) in ligand binding/discrimination (Peeters et al. 2011; also see Chap. 4 volume 2).

2.7.2 PBANR Extracellular Domains

To elucidate the structural determinants governing PBANR activation, Choi et al. (2007) generated a series of chimeric GPCRs that swapped the extracellular domains of HelzePBANR and the *D. melanogaster* pyrokinin receptor 1 (DromePKR1; analogous to DHR), which is ~100-fold less responsive to PBAN. Ligand discrimination was found to largely reside in ECL3, and to a lesser extent the N terminus (Choi et al. 2007), two domains that have been implicated in peptide ligand-GPCR interactions (Gether 2000; Gether et al. 2002; Peeters et al. 2011). Impaired activity following a swap of the respective ECL2 domains was attributed to disruption of the disulfide linkage connecting ECL2 and TM3 that is critical for GPCR folding and ligand binding (de Graaf et al. 2008). To further explore the role of HelzePBANR ECL3 in ligand discrimination, three separate point mutations were later made to residues (G297, S300, and F303) with functional groups that could potentially interact with a peptide ligand (Fig. 8.4a). Alanine substitution of S300 and F303 reduced the efficiency of Ca^{2+} mobilization compared to non-mutated controls in response to PBAN stimulation, suggesting that both residues may comprise potential contact points or contribute to the overall stabilization of the ligand binding pocket (Choi and Jurenka 2010). The role of *N*-glycosylation, which has been linked with efficient cell surface trafficking (Duvernay et al. 2005), was also examined within the context of HelzePBANR-mediated Ca^{2+} influx (Choi et al. 2007). Glutamine substitution of two consensus *N*-glycosylation sites (N19 and N22) in the HelzePBANR N terminus (Fig. 8.4a) negatively impacted PBAN-stimulated Ca^{2+} influx, an effect that was attributed to disruption of forces stabilizing the overall HelzePBANR structure (Choi et al. 2007). However, it is unclear what kind of effect, if any, the substitutions had on receptor trafficking. Deletion of the first 27 residues from the BommoPBANR N terminus, which likewise has two consensus *N*-glycosylation sites (N18 and N21), had no effect on receptor trafficking, ligand binding, or ligand-induced internalization (Hull et al. 2011). This variation in responses may be an artifact of the different assays used to assess functionality, or could reflect intrinsic differences between the respective receptors as *N*-glycosylation effects on GPCR trafficking and activity have been reported to be receptor-dependent (Duvernay et al. 2005).

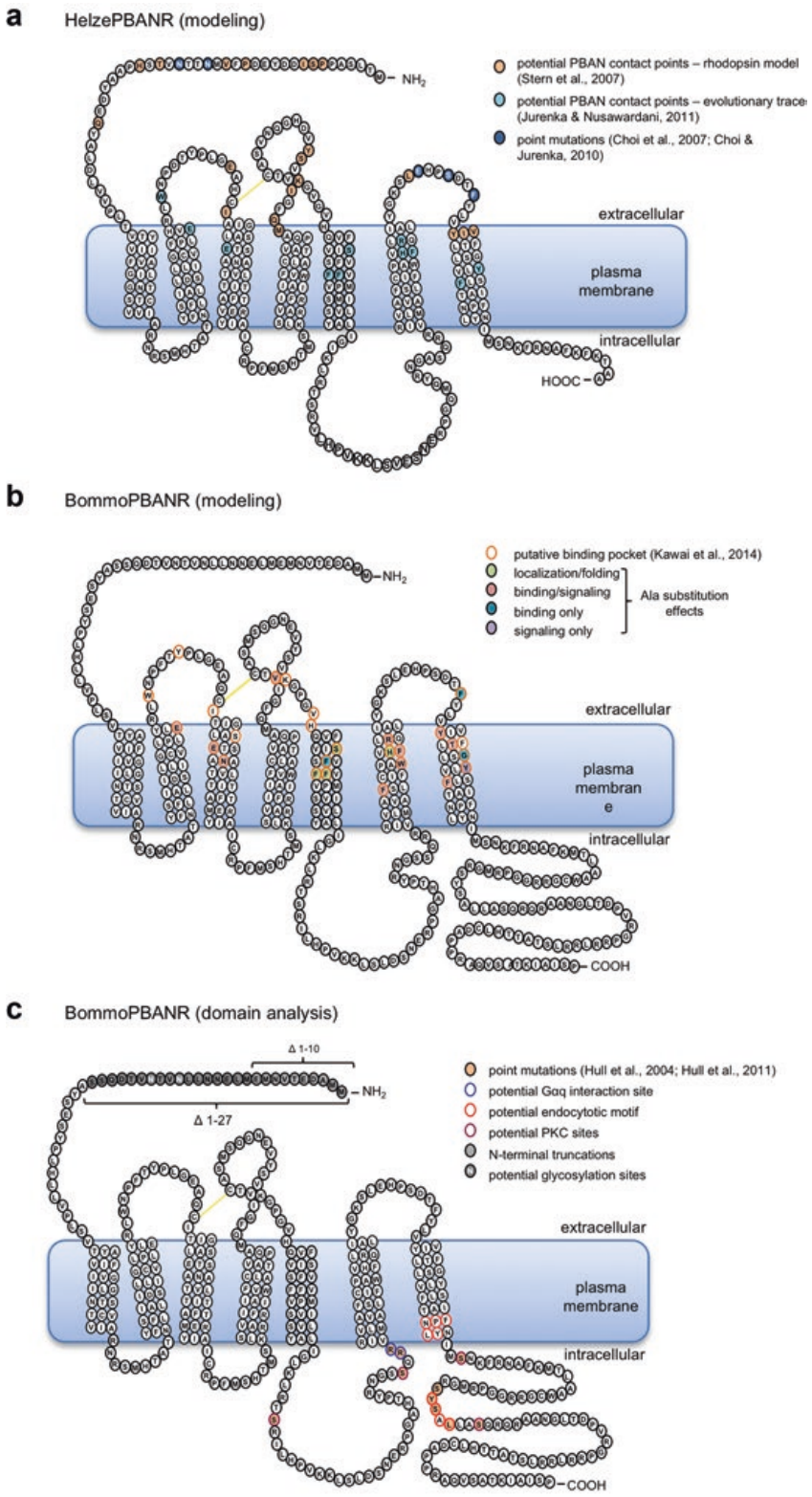

Fig. 8.4 Schematic illustration of sites in *Helicoverpa zea* and *Bombyx mori* PBANRs. (**a**) Residues predicted to comprise the PBAN ligand-binding pocket in HelzePBANR. (**b**) Residues predicted to comprise the PBAN ligand-binding pocket in BommoPBANR. (**c**) Schematic illustration of sites in BommoPBANR-C that have undergone functional analysis via site-directed mutagenesis

2.7.3 HelzePBANR Ligand Pocket

Although rhodopsin (a light sensitive GPCR) is an imperfect template for modeling peptide GPCRs (Sabio et al. 2008; Mobarec et al. 2009; Congreve et al. 2011) it can offer structural insights into potential regions of ligand contact (Congreve et al. 2011). Using molecular docking techniques with a PBAN analog (YFSPRL-NH_2) and a sequence optimized HelzePBANR conformation that utilized coordinates from the bovine rhodopsin crystal X-ray structure, Stern et al. (2007) identified twenty amino acid residues that potentially comprise the ligand binding pocket (Fig. 8.4a). This in silico HelzePBANR structure was also used to evaluate the conformational effects of the ECL swaps (see Sect. 2.7.2) between HelzePBANR and DromePKR1 (Choi et al. 2007). In that evaluation, each domain swap reduced the number of putative ligand contact points. The largest reduction was observed with the ECL2 swap, an effect that likely resulted from misorientation of the cysteines composing the ECL2-TM3 disulfide bridge (Choi et al. 2007). In a complementary approach, Jurenka and Nusawardani (2011) used an evolutionary trace method that mapped conserved residues to a three-dimensional model of HelzePBANR to identify sites critical for ligand selection and binding. The authors of that study further refined their predictions based on the presupposition that the spatial coordinates of GPCR binding domains are frequently evolutionarily conserved. Overall, they identified eleven TM residues potentially comprising a conserved FxPRL-NH_2 binding domain (Fig. 8.4a). They also suggested that the charged residues in HelzePBANR ECL3 (K294, E297, and D301) could potentially contribute to the ligand specificity revealed in the ECL3 domain swap between HelzePBANR and DromePKR1 (Choi et al. 2007), which is consistent with previous reports that ECL3 is involved in ligand specific conformational changes (Gether 2000; Gether et al. 2002; Peeters et al. 2011). However, mutagenesis analysis of E297 had little effect on receptor activity (Choi and Jurenka 2010). Because of the different in silico approaches, the two HelzePBANR models that were developed yielded different aspects of the potential binding pocket. The structural approach focused on the potential role of the ECLs, whereas the evolutionary trace approach focused on identifying the conserved GPCR binding pocket bounded by the TM helices. Taken together, the approaches identified a number of potential ligand interaction points that still await experimental verification.

2.7.4 BommoPBANR Ligand Pocket

In a separate in silico study (Kawai et al. 2014), coordinates based on crystal structures for two class A GPCRS (human β2 adrenergic receptor and human A_{2A} adrenergic receptor) facilitated identification of twenty-seven potential ligand interaction sites in BommoPBANR (Fig. 8.4b). Only three of the twenty potential residues implicated in the rhodopsin-based HelzePBANR structure (Stern et al. 2007) were identified in the BommoPBANR model. However, all of the contact points predicted by the evolutionary trace method (Jurenka and Nusawardani 2011) were

present. Sequential Ala-substitution of the residues revealed roles in ligand binding, receptor activation (i.e. mobilization of extracellular Ca^{2+}), and cell surface trafficking/protein stability. Given their interhelical localization, the four residues (S207, F211, F212, and H284) that affected cell surface expression are predicted to contribute to stabilization of the TM helical bundle. Consequently, the impaired expression observed by the authors was likely the result of receptor misfolding. Kawai and co-workers (2014) further reported a reduction in both ligand binding and receptor activation following Ala-substitution of eleven residues (E95, E120, N124, V195, F276, W280, F283, R287, Y307, T311 and F319), whereas three residues (F209, F303, G315) were implicated in ligand binding alone, and a single residue (Y318) in receptor activation. In this last case, Ala-substitution generated a mutant that exhibited normal ligand binding but impaired receptor activation, suggesting that it may be crucial in the PBAN-induced conformational change that converts the receptor from the non-activated to the activated state. Furthermore, the defects observed with five of the putative binding sites (F212, F276, W280, F283, and F319) may not be exclusively related to ligand binding as they are highly conserved in class A GPCRs and may function in the receptor conformational switch (Holst et al. 2010).

Molecular docking simulations using the BommoPBANR structure and a 5-aa FSPRL-NH_2 analog identified a number of receptor-ligand interactions largely localized to the TM bundle (Kawai et al. 2014). Similar simulations using a NmUR model and a 5-aa analog of NmU further revealed that points of contact between the critical Leu and amide in the respective ligands and the putative binding pockets were conserved: PBANR E95/NmUR E117 (TM2), PBANR E120/NmUR E142 (TM3), PBANR F283/NmUR F313 (TM6), PBANR Y318/NmUR F345 (TM7), and PBANR F319/NmUR Y346 (TM7). The Glu residues in TM2 and TM3 appear to be critically important for ligand binding among the NmUR clade of receptors, as conservation of those sites in other class A GPCRs is more limited (Kawai et al. 2014).

While the ligand-binding pocket described by Kawai and co-workers (2014) is sufficient to accommodate the C-terminal FSPRL-NH_2 active core, steric hindrance precludes it from accepting the full-length 33-aa peptide, suggesting that the non-essential N-terminal portion of PBAN interacts with the ECLs. These interactions could potentially contribute to the stabilization of ligand binding as well as serve as a selectivity filter for differentiating between ligands with similar active cores (i.e. PBAN *vs* DH). In support of this model, two ECL residues (V195 in ECL2 and F303 in ECL3) important in binding the 10-aa PBAN analog were not identified as contact points for the 5-aa analog (Kawai et al. 2014). Furthermore, FxPRL-NH_2 ligand discrimination has been demonstrated experimentally (Homma et al. 2006; Stern et al. 2007; Watanabe et al. 2007; Hariton-Shalev et al. 2013; Nusawardani et al. 2013; Shalev and Altstein 2015) and when functionally important residues in BommoPBANR and BommoDHR are compared, all are conserved with the exception of V195 (Glu in DHR) and F303 (Pro in DHR).

2.7.5 PBANR Intracellular Domains

In contrast to the ligand binding functions of the ECL domains, the C terminus and intracellular loops (ICLs) are critical for propagation and termination of the ligand signal. Ligand binding promotes G-protein dissociation, activation of downstream signal transduction cascades, and subsequent negative feedback regulation/desensitization of the activated GPCR, typically effectuated via endocytotic removal of the receptor from the cell surface (Ferguson 2001; Kristiansen 2004). Knowledge of the specific structural motifs within GPCRs (insect GPCRs in particular) that mediate these processes, however, is limited. Structure-function studies have begun to address this deficiency by providing insights into the mechanisms underlying propagation of the PBAN signal.

2.7.6 G Protein-Coupling

Pheromonogenesis is dependent on an influx of extracellular Ca^{2+} (Jurenka et al. 1991a; Choi and Jurenka, 2004, 2006; Rafaeli 2009; see Sect. 3.2.1). In *B. mori*, this event is mediated by receptor dissociation of a Gαq heterotrimeric G protein (Hull et al. 2010). Receptor-G protein coupling frequently involves ionic interactions between cationic residues near TM6 of the receptor and anionic residues in the C terminus of the G protein (Yang et al. 2002; Kleinau et al. 2010). Alignment of PBANRs with other NmU-clade GPCRs revealed a dibasic site (R263 and R264 in BommoPBANR, see Fig. 8.4c) at this junction (Hull et al. 2011). Ligand-induced internalization, a cellular event that occurs downstream of receptor activation, was significantly reduced following site-directed mutagenesis of these residues (Hull et al. 2011). The disruption in internalization suggests that PBANR signaling was impacted, providing indirect evidence for this region in PBANR-G protein coupling.

2.7.7 C-Terminal Motifs Critical for Ligand-Induced Internalization

A number of conserved C-terminal motifs play critical roles in GPCR desensitization and endocytosis (Ferguson 2001; Kristiansen 2004). The C-terminal region of BommoPBANR has two such motifs, NPxxY (residues 325–329) and YxxΦ (residues 360–363) (Fig. 8.4c). Although NPxxY has been reported to function in the internalization of multiple vertebrate GPCRs (Barak et al. 1995; Gripentrog et al. 2000; He et al. 2001; Bouley et al. 2003), its role in endocytosis is receptor dependent (Slice et al. 1994; Hunyady et al. 1995). The YxxΦ motif (Y = Tyr, x = any amino acid, and Φ = amino acid with a bulky hydrophobic sidechain) has also been implicated in ligand-induced internalization (Paing et al. 2004; Pandey 2009) and is present in the C terminus of numerous peptide GPCRs. Using a series of C-terminal truncations, the BommoPBANR internalization motif was mapped to a 10-aa region spanning residues 357–367, which contain the YxxΦ motif (Hull et al. 2005).

Ala-substitution of the critical residues in the motif likewise impaired internalization (Hull et al. 2005), albeit not to the same extent as the C-terminal truncation, which suggests that, similar to other receptors (Johnson et al. 1990; Nussenzveig et al. 1993; Thomas et al. 1995), the PBANR endocytotic mechanism utilizes multiple signals. The C-terminal YxxΦ motif, YSAL, is highly conserved among the lepidopteran PBANRs and a number of related receptors (i.e. PKR2) in other species, but has diverged in DHRs (YTAM/V), and is not readily apparent in PKR1. This variance suggests that regulation of those receptors either utilizes a different internalization signal or proceeds via a non-endocytotic pathway. Whether or not this sequence is sufficient in and of itself to promote internalization of PBANRs from other species has yet to be experimentally determined.

2.7.8 Phosphorylation-Dependent Internalization of BommoPBANR

Desensitization and internalization of GPCRs are triggered in response to ligand-induced phosphorylation of sites in the ICLs and/or C terminus by G protein-coupled receptor kinases (GRKs) and/or second messenger-dependent kinases, such as protein kinase C (PKC) (Ferguson 2001; Kristiansen 2004). Consistent with this paradigm, BommoPBANR internalization was blocked by the general kinase inhibitor staurosporine (Hull et al. 2005) and significantly impaired following double Ala-substitution of two consensus PKC sites in the BommoPBANR C terminus, S333 and S366 (Hull et al. 2011). In support of PKC-mediated phosphorylation as an internalization trigger, RNAi knockdown of endogenous PKC in Sf9 insect cells also blocked PBANR internalization (Hull et al. 2011). Furthermore, localization of S366 within the 10-aa region (i.e. residues 357–367) critical for ligand-induced internalization (Fig. 8.4c) and the incomplete blockage of internalization following Ala-substitution of the YxxΦ motif are consistent with S366 functioning as a pivotal site for PBANR internalization. Sequence alignments have shown that both the S333 and S366 PKC sites are highly conserved in other PBANRs, which may indicate that feedback regulation of this class of receptors is evolutionarily conserved. Although PKC sites are predicted in the C terminus of most DHRs, the S366 site has not been conserved, providing additional evidence that DHR regulation may proceed via a different pathway.

3 PBAN Signal Transduction

The driving element of numerous studies over the years has been to elucidate the molecular basis underlying conversion of the external PBAN signal into the biological response of pheromone production and release. Initial studies sought to unravel the complex signaling interconnections by examining the effects of various pharmacological compounds (both inhibitors and activators) on pheromonogenesis. While data generated using these compounds can be ambiguous given the possibility of non-specific pharmacological effects and target specificity that varies with

concentration (e.g. NaF at 10 mM acts as a phosphatase inhibitor but at 1–2 mM acts as a G protein activator), it can provide insights into potential mechanisms. Advances in molecular techniques, in particular the applicability of RNAi, have provided additional tools to decipher the molecular components underlying the PBAN signaling cascade. This cascade, which has been most extensively elucidated in heliothine moths (*H. zea*, *H. virescens*, and *H. armigera*) as well as *B. mori*, is now thought to diverge depending on the step in the biosynthetic pathway that is ultimately activated (i.e. early step *vs* late step).

3.1 G Protein Activation

The initial step in most extracellular signal transduction cascades requires dissociation of heterotrimeric guanine nucleotide binding proteins (i.e. G proteins α, β, and γ) from cell surface receptors and subsequent activation of downstream effectors (Cabrera-Vera et al. 2003). Receptor association/dissociation is dependent on the guanine nucleotide binding and hydrolysis activity of Gα subunits, which have been classified based on sequence variation and effector pathways activated into five subtypes: Gαs (stimulate cAMP production), Gαi/o (inhibit cAMP production), Gαq (stimulate Ca^{2+} influx), $G\alpha_t$ (phototransduction), and $G\alpha_{12/13}$ (actin cytoskeletal remodeling) (Cabrera-Vera et al. 2003; Meigs and Lyakhovich 2012). Prior to PBANR identification, PBAN-induced elevation of cAMP levels (Rafaeli and Soroker 1989; also see Sect. 3.3.1) and the pheromonotropic effects of NaF (1–2 mM) on isolated PGs (Rafaeli and Gileadi 1996a) suggested the involvement of G proteins in the PBAN signal transduction cascade. Using homology-based cloning and genomic mining methods, transcripts for four Gα subunits (two Gαs, a Gαo, and a Gαq) were amplified from *B. mori* PGs (Hull et al. 2007a, 2010). Sequential RNAi knockdown of the four Gα subunits revealed that only Gαq had a role in transmitting the PBAN pheromonotropic signal (Hull et al. 2010).

3.2 PBAN-Induced Influx of Ca^{2+}

3.2.1 Essential Role of Extracellular Ca^{2+}

Initial studies using isolated PGs from diverse moth species demonstrated that the pheromonotropic effects of PBAN require extracellular Ca^{2+} (Jurenka et al. 1991a; Fónagy et al. 1992d; Jurenka et al. 1994; Ma and Roelofs 1995; Matsumoto et al. 1995b; Soroker and Rafaeli 1995; Zhao et al. 2002; Choi and Jurenka 2004, 2006; Hull et al. 2007a). Moreover, pharmacological manipulation (e.g. ionomycin, A23187, or thapsigargin) of intracellular Ca^{2+} levels could trigger pheromone production (Jurenka et al. 1991a; Fónagy et al. 1992c, d; Rafaeli 1994; Jurenka et al. 1994; Ma and Roelofs 1995; Matsumoto et al. 1995a, b; Soroker and Rafaeli 1995;

Rafaeli and Gileadi 1996a; Zhao et al. 2002; Hull et al. 2007a), whereas inorganic Ca^{2+} channel blockers inhibited pheromone production (Jurenka et al. 1991a; Fónagy et al. 1992d; Ma and Roelofs 1995; Matsumoto et al. 1995b; Choi and Jurenka 2004). Taken together, these findings provided indirect evidence for PBAN-dependent opening of cell surface ion channels and the concomitant influx of Ca^{2+}. Subsequent advances in fluorescent Ca^{2+} imaging techniques provided direct evidence for the rise in intracellular Ca^{2+} in response to PBAN binding in isolated *H. zea* and *B. mori* PGs (Choi and Jurenka 2006; Hull et al. 2007a).

3.2.2 Identification of the PBAN-Activated Ca^{2+} Channels

The most pervasive Ca^{2+}-permeable ion channels in cells are voltage-operated channels (VOCs) (Lacinova 2005) and receptor-activated Ca^{2+} channels (RACCs) (Prakriya and Lewis 2015; Redondo and Rosado 2015), which include diacylglycerol (DAG)-dependent channels and store-operated channels (SOCs). Consistent with the early prediction of receptor involvement, VOC blockers had no effect on pheromone production in *H. zea* (Jurenka et al. 1991a; Choi and Jurenka 2006) or *B. mori* (Hull et al. 2007a), whereas SKF-96365, an inhibitor of both VOC and RACC, had pronounced pheromonostatic effects in *H. virescens* and *B. mori* (Jurenka 1996; Hull et al. 2007a). Further pharmacological manipulation of channel activity using inhibitors/activators of SOCs suggested that PBAN signals through an SOC pathway rather than a DAG-dependent channel (Hull et al. 2007a).

For many systems, the SOC pathway consists of stromal interaction molecule 1 (STIM1) functioning as a Ca^{2+} sensor and Orai1 as the pore-forming unit of the channel (López et al. 2016). Consistent with a role in the PBAN-activated SOC pathway, targeted knockdown of *B. mori* homologs of STIM1 and Orai1 negatively affected pheromone production without affecting non-pheromonotropic enzyme activities (Hull et al. 2009). The dependence on extracellular Ca^{2+} in PBAN-regulated pheromone pathways and the presence of STIM1 and Orai1 transcripts in moth PG transcriptomes (Ding and Löfstedt 2015) suggests that the STIM1-Orai1 SOC pathway is likely conserved in moths.

3.3 Role of Other Second Messengers

3.3.1 cAMP

While extracellular Ca^{2+} has been shown to be an absolute requirement for pheromonotropic activity in every moth species studied to date, the role of cAMP in the PBAN signal cascade appears to be species-dependent. Early cAMP radioimmunoassays demonstrated a PBAN-mediated increase of cAMP levels in isolated *H. armigera* PGs (Rafaeli and Soroker 1989; Rafaeli 1994; Soroker and Rafaeli 1995; Rafaeli and Gileadi 1996a). Furthermore, pharmacological manipulation (e.g.

cAMP analogs, phosphodiesterase inhibition, or adenylate cyclase activation) of PG cAMP levels promoted pheromone production in *H. armigera* (Rafaeli and Soroker 1989; Soroker and Rafaeli 1995; Rafaeli and Gileadi 1996a), *H. zea* (Jurenka et al. 1991a), *H. virescens* (Jurenka 1996), and *Argyrotaenia velutinana* (Jurenka et al. 1994). In contrast, similar studies failed to find cAMP-linked pheromonotropic effects in *B. mori* (Fónagy et al. 1992d), *S. litura* (Matsumoto et al. 1995b), or *O. nubilalis* (Ma and Roelofs 1995) and no evidence was found of PBAN-mediated cAMP elevation in *B. mori* PGs (Hull et al. 2007b). There is, however, a strong correlation between this second messenger event and the pheromone biosynthetic activity under PBAN control. In species that utilize cAMP, the pheromonotropic control point resides in fatty acid biosynthesis, most likely the acetyl-CoA carboxylase (Tang et al. 1989; Jurenka et al. 1991b; Tsfadia et al. 2008). However, in species that do not undergo cAMP elevation, PBAN regulates a step(s) further along in the biosynthetic pathway, usually fatty acyl reduction (Fabriàs et al. 1994; Ma and Roelofs 1995; Ozawa et al. 1995; Ozawa and Matsumoto 1996; Moto et al. 2003; Eltahlawy et al. 2007) and, *in B. mori*, a second step involving cytoplasmic lipid droplet lipolysis (Fónagy et al. 2000; Ohnishi et al. 2006). While the evidence is currently too limited to draw broad conclusions regarding the relationship between cAMP signaling and PBAN regulation, the predictable associations suggest an avenue of potential research, in particular within species (*Thaumetopoea pityocampa*, *M. sexta*, *Sesamia nonagrioides*) in which the pheromonotropic control point is known be a step late in biosynthesis (Fabriàs et al. 1995; Fang et al. 1995; Mas et al. 2000) or species (*Ostrinia furnacalis*, *M. brassicae*, *Dendrolimus punctatus*, *P. separata*) where PBAN regulates a step in the fatty acid pathway (Jacquin et al. 1994; Zhao and Li 1996; Zhao et al. 2002; Fónagy et al. 2011; Köblös et al. 2015). It would likewise be interesting to examine the role of the PBANR variants in the contrasting signal transduction cascades. Jurenka and Rafaeli (2011) proposed that structural variations in the C-terminal lengths of the PBANR variants may contribute to the differing downstream responses with shorter C-terminal tail PBANRs linked to cAMP dependent pathways and the longer C-terminal PBANRs linked to Ca^{2+} influx alone.

3.3.2 IP_3

Similar to Ca^{2+} and cAMP, the phosphoinositide IP_3 (inositol 1, 4, 5-triphosphate) is a signal transduction messenger. IP_3 is generated from phospholipase C (PLC)-mediated hydrolysis of PIP_2 (phosphatidylinositol-4,5-bisphosphate) in response to receptor activation and typically functions in the propagation of receptor-mediated Ca^{2+} signaling by mobilizing intracellular Ca^{2+} stores (Balakrishnan et al. 2015). An early study on the PBAN mode of action reported that pheromonotropic activity of *H. armigera* PGs was reduced following pharmacological depletion of IP_3 (Rafaeli 1994). A later study in *B. mori* reported that total inositol phosphate levels in isolated PGs rose in response to PBAN and that RNAi knockdown of a putative IP_3 receptor suppressed pheromone production (Hull et al. 2010). These findings

implicated PBANR-mediated activation of PLC. In support of this, pharmacological inhibition of PLC activity with either U73122 or compound 48/80 negatively impacted pheromone production in *B. mori*, whereas the inactive analog of U73122 had no effect (Hull et al. 2010). The pheromonostatic effects of compound 48/80, however, differed from a previous study that found no effect on *B. mori* pheromone production (Matsumoto et al. 1995a). Given that the preponderance of evidence available with the more recent study strongly pointed to PLC activity, the contrasting result was attributed to methodological differences. Separate studies demonstrating the critical importance of SOC components STIM1 and Orai1 (see Sect. 3.2.2 and Hull et al. 2009) in pheromone production likewise implicated PLC activity.

3.4 PBAN-Mediated PLC Activity

PCL-dependent activation of SOCs is predominantly driven by PLCβ and PLCγ (Drin and Scarlata 2007). PLCβ is generally activated downstream of GPCRs (Drin and Scarlata 2007), whereas PLCγ functions downstream of tyrosine kinase and non-receptor tyrosine kinases (Patterson et al. 2005). Using genomic mining methods, PLCβ1, PLCβ4, and PLCγ transcripts were amplified from *B. mori* PGs (Hull et al. 2010). Consistent with the expected signaling paradigm, RNAi-mediated knockdown of PLCβ1 significantly reduced pheromone production. PLCγ knockdown likewise mitigated the pheromonotropic effects of PBAN (Hull et al. 2010). Based on findings in other systems (Patterson et al. 2005), PLCγ was postulated to function in PBAN signaling as a molecular scaffold that stabilizes the protein-protein interactions essential for formation of the SOC complex rather than catalyzing PIP_2 hydrolysis.

3.5 Signal Transduction Post-PBAN-Mediated Ca^{2+} Influx

3.5.1 Calmodulin

As discussed above, the role of cAMP in PBAN signaling appears to differentiate the enzymatic step in the respective sex pheromone biosynthetic pathways under PBAN control. The GPCR-mediated generation of cAMP can be an indication that the receptor couples through Gαs, which stimulates adenylate cyclase activity following receptor dissociation. However, cAMP production in *H. armigera* reportedly occurred downstream of Ca^{2+} influx (Soroker and Rafaeli 1995), suggesting the involvement of a Ca^{2+}-dependent adenylate cyclase. Additional pharmacological profiling of the PBAN cascade revealed that inhibition of calmodulin, a multifunctional Ca^{2+} binding protein that interacts with diverse proteins, blocked the PBAN-mediated increase of cAMP in *H. armigera* (Rafaeli and Gileadi 1996a) and

mitigated the pheromonotropic effects of PBAN in *H. armigera* (Soroker and Rafaeli 1995) as well as *S. litura* and *B. mori* (Matsumoto et al. 1995a, b; Ozawa and Matsumoto 1996). In support of these results, a calmodulin homolog identical to the *D. melanogaster* protein was purified from *B. mori* PGs (Iwanaga et al. 1998). Among the enzymatic activities reportedly mediated by Ca^{2+}-bound calmodulin are adenylate cyclases (Halls and Cooper 2011), suggesting that the Ca^{2+}-dependent increase in cAMP observed in heliothine moths is likely driven by one of these cyclases. Because many calmodulin interacting proteins are directly or indirectly involved in protein phosphorylation, the results observed in *S. litura* and *B. mori*, neither of which utilizes cAMP in PBAN signaling, may be attributable to impaired phosphorylation cascades.

3.5.2 Kinase Activity

GPCR-mediated activation of biosynthetic pathway enzymes typically involves a phosphorylation cascade driven by diverse kinase (phosphorylation) and phosphatase (dephosphorylation) steps. The generation of cAMP, the critical role of calmodulin, and the importance of PKC in feedback regulation of BommoPBANR in vitro (see Sect. 2.7.8) strongly suggested kinase activity in PBAN signaling. While early studies assessing the effect of both broad spectrum and specific kinase inhibitors found no effect on pheromone production in either *B. mori* (Matsumoto et al. 1995a) or *H. armigera* (Soroker and Rafaeli 1995), the PKC activator, phorbol 12-myrstate 13-acetate (PMA), was found to have pheromonotropic activity in *H. armigera* (Soroker and Rafaeli 1995). This effect, however, did not extend to *B. mori* or *S. litura* (Matsumoto et al. 1995b; Ozawa et al. 1995). A more recent study using anti-phosphoamino acid antibodies found clear evidence of PBAN-mediated phosphorylation in *B. mori* (Ohnishi et al. 2011). Furthermore, RNAi-mediated knockdown of a Ca^{2+}-bound calmodulin dependent kinase II (CaMKII) in *B. mori* PGs reduced PBAN-induced pheromone production and diminished phosphorylation of a critical lipid droplet-associated protein, whereas knockdown of putative protein kinase A (PKA) and PKC transcripts had no effect (Ohnishi et al. 2011).

3.5.3 Phosphatase Activity

In contrast to the early kinase inhibitor studies, pharmacological inhibition of phosphatase activity had pronounced pheromonostatic effects in *B. mori* (Matsumoto et al. 1995a, b; Ozawa and Matsumoto 1996; Fónagy et al. 1999) as well as *H. zea* and *H. virescens* (Jurenka 1996). Inhibition of ionophore-induced pheromone production in *H. zea* suggested that phosphatase activity occurs downstream of Ca^{2+} influx (Jurenka 1996), thus ruling out an effect similar to LiCl, which inhibits IP_3 generation. The effectiveness of inhibitors specific for calcineurin (Fónagy et al.

1999), a protein phosphatase b activated by Ca^{2+}-bound calmodulin, was consistent with previous studies demonstrating calmodulin activity. In support of this role, both calcineurin subunits were amplified from *B. mori* PGs (Yoshiga et al. 2002). Determination of the rate-limiting steps in heliothine moths and *B. mori* suggest that calcineurin or calcineurin-like phosphatase activity comprises the penultimate control point in PBAN signaling. In heliothine moths, PBAN activates acetyl-CoA carboxylase, the critical point in fatty acid biosynthesis that catalyzes carboxylation of acetyl-CoA to yield malonyl-CoA. In *B. mori* (and other moths), PBAN regulates a fatty acyl reductase that shares biochemical characteristics with HMG-CoA reductase (Ozawa et al. 1995). In both cases (i.e. acetyl-CoA carboxylase and HMG-CoA reductase), enzymatic activity is phosphorylation-dependent (Zammit and Easom 1987; Brownsey et al. 2006).

3.6 Model of Pheromone Regulation by PBAN Signaling

Based on diverse studies spanning more than 20 years (many of which were briefly described above), a model for the molecular signaling cascade underlying PBAN-mediated regulation of pheromone production has emerged (Fig. 8.5). Circadian activation of extero-receptors and brain hormones such as allatotropins/allatostatins that influence JH biosynthesis (Cusson and McNeil 1989; Woodhead et al. 1989; Picimbon et al. 1995; Stay and Tobe 2007) may have a role in PBAN release into the hemolymph where it interacts with PBANRs localized at the plasma membrane of PG cells. The ensuing conformational change in PBANR results in dissociation of the heterotrimeric G protein complex with subsequent Gαq activation of PLCβ1-mediated hydrolysis of PIP2 into DAG and IP_3. The soluble IP_3 diffuses through the cytosol to activate IP_3 receptors in the endoplasmic reticulum (ER) membrane, which promotes release of stored Ca^{2+}. The drop in luminal Ca^{2+} levels results in translocation of STIM1 to the plasma membrane where it triggers an influx of extracellular Ca^{2+} through Orai1 channels, presumably via interactions with a scaffolding complex that includes PLCγ. The concomitant rise in intracellular Ca^{2+} allows for formation of Ca^{2+}-calmodulin complexes, at which point the pathway exhibits species-dependent divergence. In heliothines and species that utilize cAMP, the Ca^{2+}-calmodulin complexes stimulate adenylyl cyclase activity. The rise in cAMP then drives a cascade culminating in activation of the fatty acid biosynthetic pathway enzyme, acetyl CoA-carboxylase. In *B. mori,* and presumably species in which PBAN regulates a step late in pheromonogenesis, the Ca^{2+}-calmodulin complexes activate both calcineurin (a protein phosphatase) and calmodulin-dependent kinase II (CamKII). Calcineurin in turn activates fatty acyl reductase, the terminal step in pheromone biosynthesis, while CamKII-dependent phosphorylation of lipid storage droplet protein-1 promotes lipolytic release of stored pheromone precursors (Fig. 8.5).

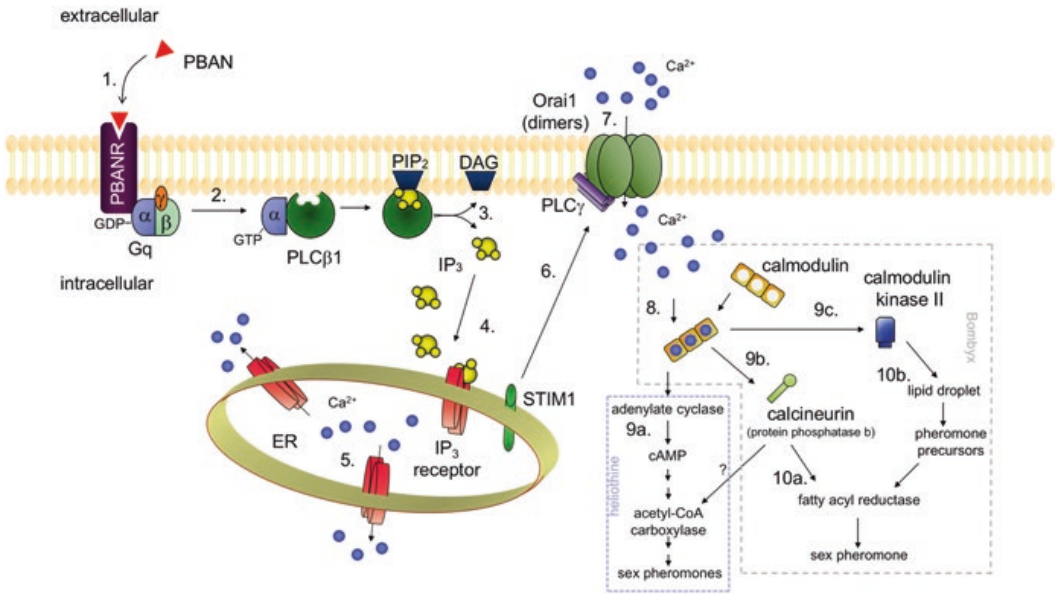

Fig. 8.5 Proposed PBAN signal transduction cascade. (*1*) PBAN circulating in the hemolymph binds to PBANR in the plasma membrane of PG cells. (*2*) PBAN binding promotes dissociation of Gαq from PBANR with subsequent activation of PLCβ1. (*3*) PLC-mediated hydrolysis of PIP_2 yields DAG and soluble IP_3. (*4*) Cytosolic IP_3 interacts with IP_3 receptors in the ER membrane. (*5*) Activation of IP_3 receptors promotes release of stored Ca^{2+}. (*6*) The reduction in ER luminal Ca^{2+} levels promotes interactions between STIM1 and Orai1 channels in the plasma membrane. The resulting complex formation may be stabilized by protein-protein interactions with SH3 domains in PLCγ. (*7*) The activated Orai1 channels open allowing an influx of extracellular Ca^{2+}. (*8*) Free calmodulin complexes with the intracellular Ca^{2+}. (*9a*) In heliothines, the Ca^{2+}-calmodulin complex stimulates adenylate cyclase activity and the production of cAMP, which subsequently initiates a protein kinase A/C phosphorylation cascade. PBAN signaling culminates in activation of acetyl-CoA carboxylase, the limiting step in fatty acid biosynthesis. Given evidence in the literature that this enzyme is activated in response to dephosphorylation and that pharmacological inhibition of phosphatase activity in *H. zea* and *H. virescens* has pheromonostatic effects, it is likely that a protein phosphatase, possibly calcineurin, may function in acetyl-CoA carboxylase activation. (*9b*) In *B. mori*, calcineurin is activated by the Ca^{2+}-calmodulin complex, which also activates (*9c*) a calmodulin-dependent protein kinase II (CamKII). (*10a*) CamKII phosphorylates a lipid droplet storage protein critical for lipolytic release of pheromone precursors stored in cytosolic lipid droplets. (*10b*) Calcineurin dephosphorylates fatty acyl reductase, the terminal enzymatic reaction in the *B. mori* pheromone biosynthetic pathway. Abbreviations: *cAMP* cyclic adenosine 3′, 5′-monophosphate, *DAG* diacylglycerol, *ER* endoplasmic reticulum, *GDP* guanosine diphosphate, *Gq* G protein α subunit q, *GTP* guanosine-5′-triphosphate, *IP_3* inositol 1,4,5-trisphosphate, *PIP2* phosphatidylinositol (4,5)-bisphosphate, *PLC* phospholipase C, *STIM1* stromal interaction molecule 1

4 Targeted Disruption of PBAN Pathway

Current integrated pest management strategies that focus on mating disruption frequently exploit synthetic pheromone blends (Witzgall et al. 2008; El-Sayed et al. 2009). However, for species that utilize multi-component pheromone blends with cost prohibitive chemistries, targeted disruption of pheromone biosynthetic

pathways has significant potential as an alternative control measure. This is the case for the black cutworm moth, *A. ipsilon*, a polyphagous, polyandrous pest with multi-continental populations and intra-specific genetic variations (Wakamura et al. 1986; Picimbon et al. 1995, 1997; Gadenne et al. 1997; Duportets et al. 1998; Gemeno and Haynes 1998; Gemeno et al. 2000; Du et al. 2015). Insect GPCRs in particular have been proposed as promising targets for the next generation of insecticides (Scherkenbeck and Zdobinsky 2009; Van Hiel et al. 2010; Bai and Palli 2013; Grimmelikhuijzen and Hauser 2013; Audsley and Down 2015). This interest has driven significant efforts in developing peptidomimetics that overcome limitations (i.e. environmental instability, poor cuticular penetrance, and susceptibility to proteolytic degradation in the hemolymph) inherent to peptides that make them unsuitable for pest management. Because this topic has been extensively reviewed elsewhere (Altstein 2001, 2004b; Nachman et al. 2009a; Scherkenbeck and Zdobinsky 2009), we provide only a brief overview of some of the most intriguing developments.

4.1 Peptidomimetics

4.1.1 PBAN Agonists

PBAN agonists, small molecules that activate the receptor in the absence of the endogenous ligand, provide valuable insights into the structural requirements and chemistries crucial for ligand binding and cuticular penetration. In addition, they offer possibilities in pest management as continuous pheromonogenic stimulation via a bound agonist could lead to pheromone release asynchronous with male mating behaviors and/or depleted pheromone. Early peptide engineering studies revealed that modification of the terminal Phe in the pentapeptide FTPRL-NH_2 with a hydrophobic cage-like *o*-carborane moiety (a cluster composed of boron, carbon, and hydrogen), 1-pyrenebutyric acid, 9-fluoreneacetic acid, or 2-amino7-bromofluorene yielded topically active pheromonogenic analogs with enhanced cuticular penetrance and greater hemolymph persistence (Nachman et al. 1996; Teal and Nachman 1997, 2002). Additional studies incorporating β-amino acids further highlight the importance of the Phe residue for pheromonotropic activity (Nachman et al. 2009a).

4.1.2 PBAN Antagonists

The structural, conformational and dynamic features of agonists can serve as the basis for rational design of antagonists, which require the compound to bind the receptor without activating the signal transduction cascade. Replacing the Thr in the pheromonogenic septapeptide RYFTPRL-NH_2 with D-Phe yielded a linear peptide antagonist that significantly inhibited pheromone production following injection

(Zeltser et al. 2000). Backbone cyclization techniques have also yielded antagonists with pheromonostatic effects that can persist for several hours (Altstein et al. 2000). A linear RYF[dF]PRL-NH_2 analog that incorporated an aliphatic amine exhibited enhanced cuticular penetration while retaining pheromonostatic properties (Nachman et al. 2009b).

4.1.3 Receptor Selective Analogs

FxPRL-NH_2 analogs have been reported to have differing receptor effects depending on the activity assayed (e.g. melanotropic *vs* pheromonotropic) despite mediation of both activities by the same peptidergic sequence (Matsumoto et al. 1992; Altstein et al. 1996) and receptor (Zheng et al. 2007; Kim et al. 2008). Sequential D-Phe scan of a modified PBAN sequence (YFSPRL-NH_2) generated a selective antagonist that significantly reduced pheromone production with no effect on pupal melanization (Ben-Aziz et al. 2005). An amphiphilic version of the antagonist, that incorporated an aliphatic amine via succinic acid at the N terminus of the pentapeptide, retained selective antagonist properties while exhibiting enhanced cuticular penetrance (Nachman et al. 2009b). Similarly, replacement of the critical Phe with a β-homo-amino acid yielded an analog that affected melanization but had no effect on pheromone production (Nachman et al. 2009a). Incorporation of a dihydroimidazoline moiety into the FxPRL-NH_2 hexapeptide sequence likewise generated a selective melanotropic antagonist devoid of either pheromonotropic or pheromonostatic activities (Nachman et al. 2010). The selectivity observed in these peptidomimetic studies suggests that the melanotropic receptor tolerates greater conformational deviations in the ligand than the pheromonotropic receptor. This ligand selectivity is corroborated by both in vitro and in silico studies of FxPRL-NH_2 receptors that show dissimilar three-dimensional conformations, electrostatic potentials, and ligand preferences (Hariton-Shalev et al. 2013; Shalev and Altstein 2015). While the development of selective antagonists will undoubtedly provide additional insights into the development of novel pest management agents, it is apparent that despite years of study, our understanding of FxPRL-NH_2 pleiotropism at the molecular level will remain a fertile area of research.

4.2 RNAi: The New Frontier?

As a biorational approach that can be specifically tailored to individual pest species, RNAi holds great promise for the future of insect pest management (Price and Gatehouse 2008; Burand and Hunter 2013). Though still in its infancy, the viability of using transgenic plants that trigger RNAi-mediated suppression of select pest genes has been effectively demonstrated (Baum et al. 2007; Mao et al. 2007, 2011; Pitino et al. 2011). While those studies focused on the control potential associated with knockdown of diverse enzymes, current studies assessing the effects of

neuropeptide/GPCR RNAi knockdown on peptidergic regulation of insect biology (e.g. Terhzaz et al. 2007; Arakane et al. 2008; Badisco et al. 2011; Bai et al. 2011; Terhzaz et al. 2015; Zandawala et al. 2015) may provide an additional biorational set of tools for the development of next generation pest management strategies.

4.2.1 RNAi-Knockdown: PBAN

To date, RNAi-mediated knockdown of PBAN has only been reported for two species, *H. zea* (Choi et al. 2012) and *S. litura* (Lu et al. 2015). In both species, injection of double-stranded RNAs (dsRNAs) corresponding to a fragment of the respective DH-PBAN gene markedly reduced sex pheromone production. In *H. zea*, however, the PBAN dsRNA injections, which were performed using 4–5 day old female pupae, also affected adult emergence with a significantly higher percentage of injected pupae unable to eclose (Choi et al. 2012). A similar phenotype was reported in another heliothine moth following knockdown of PBAN, but not PBANR, suggesting that the failure to eclose properly may be linked to DH, which functions in termination of pupal diapause in heliothine moths (Xu and Denlinger 2003; Sun et al. 2003).

4.2.2 Genome Editing: PBAN

Advances in genome editing methodologies have extended targeted gene mutagenesis capabilities. One such approach utilizes Transcription Activator-Like Effector Nucleases (TALENs) to introduce small deletions or insertions at the gene level that cause frameshift mutations/truncations. Recently, Shiomi et al. (2015) used this method to make targeted deletions in the *B. mori* DH-PBAN gene yielding prepropeptides severely truncated within the signal peptide region precluding generation of the PBAN sequence. While the mutations clearly affected the induction of embryonic diapause, the pheromonogenic effects, which were not the focus of the study and were thus only assessed superficially, appeared to be muted with a slight reduction in the male behavioral response.

4.2.3 RNAi-Knockdown: PBANR

PBANR transcripts have been knocked-down in *B. mori* (Ohnishi et al. 2006), *P. xylostella* (Lee et al. 2011), and *H. armigera* (Bober and Rafaeli 2010). Injection of dsRNAs corresponding to a 417-nt fragment of BommoPBANR into 1-day-old pupae triggered receptor knockdown and significantly impaired sex pheromone production and disrupted lipolysis of cytoplasmic lipid droplets (Ohnishi et al. 2006). Similarly, knockdown of PluxyPBANR in pupae 1 day prior to adult emergence with dsRNAs corresponding to a 549-nt fragment resulted in a ~50% reduction in sex pheromone production and a 20–40% reduction in female mating (Lee et al.

2011). That group also reported decreased expression of two desaturases thought to be involved in the *P. xylostella* sex pheromone biosynthetic pathway following PluxyPBANR knockdown (Lee and Kim 2011). Unlike *B. mori* and *P. xylostella*, the effects of HelarPBANR knockdown were evaluated in adult male moths. An earlier study reported expression of HelarPBANR in the male aedeagus, a reproductive organ adjacent to the male abdomen through which sperm from the testis is transferred during copulation and which is usually associated with male-derived sex pheromone-like compounds (Rafaeli et al. 2007). Injection of dsRNAs corresponding to a 880-nt fragment of HelarPBANR in 1-day-old adult male *H. armigera* significantly reduced PBAN-stimulated production of male volatile compounds (Bober and Rafaeli 2010). While the relevance of these compounds in *H. armigera* mating behavior remains to be demonstrated, similar compounds have been linked to stimulation of female receptivity and inhibition of male competition (Teal and Tumlinson 1984; Kehat and Dunkelblum 1990; Huang et al. 1997; Hillier and Vickers 2004; Hillier et al. 2006). In the European corn borer, *O. nubilalis*, the male scent odor is crucial for the acceptance of the male by the female (Royer and McNeil 1992; Picimbon 1996; Farrell and Andow 2017). Regardless, the results demonstrate that in a wide variety of moths the role of PBANR functionality in pheromone biosynthesis is certainly not restricted to females and further underscores the pleiotropic nature of the receptor and its multifunctional ligand.

5 Concluding Remarks

The past 30 years have witnessed significant progress in our understanding of pheromonogenesis in moths and its neuroendocrine regulation. Interestingly, rather than clarifying our understanding of pheromonotropic control, elucidation of the "black box" has illuminated yet another layer of complexity and provided new puzzles for us to unravel.

Some of the questions raised with this new framework of entomology, chemical ecology, physiology and molecular biology research that we find the most intriguing include:

- What is the molecular basis for regulation of the pleiotropic FxPRL-NH_2 peptide/receptor system?
- How is ligand selectivity of PBANRs/DHRs achieved?
- What is the evolutionary significance of the different control points (fatty acid biosynthesis *vs* terminal modification) in the PBAN pathway, and how did this divergence arise?
- What biological role do the concomitantly expressed PBANR variants play in PBAN signaling?
- How are transcription and alternative splicing of PBANR regulated?

Undoubtedly, rapid developments in mRNA sequencing, bioinformatics, molecular engineering, and proteomics will play a significant role in resolving these new

questions. In addition, advances such as CRISPR in insect genome editing (Taning et al. 2017), and RNAi (see Chap. 5), despite the current limitations of this technology in lepidopterans (Terenius et al. 2011), can provide unequivocal demonstration of the roles calmodulin, calcineurin, and acetyl-CoA carboxylase have in heliothine pheromonogenesis and finally reveal how conserved PBAN signaling pathways function across species. Similar application of these technologies can also provide insights into the role of antagonistic peptidomimetics in receptor regulation.

Continued research into the mechanisms underlying PBANR function in moths, as well as related receptors in other species, will help answer questions regarding the biological significance of the FxPRL-NH_2 family and how alternative splicing plays a role in mediating that biology. This knowledge will provide insights into the complexities of GPCRs, and can potentially be applied towards the development of novel biorationally designed insect control agents. These fundamental studies will also continue to provide insights into mammalian endocrinology, lipid biology, and the molecular interactions underlying peptidergic binding/activation of pleiotropic GPCRs.

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Glossary

^{3}H-labeled pheromone A synthetic pheromone with a hydrogen atom exchanged by tritium (^{3}H, the radioactive isotope of hydrogen) used in pionnering biochemical studies in order to measure the pheromone concentration using the beta radiation

Acetylcholine (*Ach*) An organic neurotransmitter chemical that functions in the brain of many organisms including human and insects

Acheta domesticus (house cricket) A study model for neurogenesis in the brain

Actinobacter A genus of Gram-negative bacteria belonging to the class of Grammaproteobacteria known to occur in pairs

Active core (FxPRL-NH_2) A specific motif in the PBAN pentapeptide that is active in inducing pheromone production

Acyrthosiphon pisum (pea aphid) A sap-sucking insect in the Aphididae family

ADAR (adenosine deaminase acting on RNA) An enzyme that recognizes specific RNA duplexes and affects RNA sequence through adenosine (A)-to-Inosine (I) mutations

Aedes aegypti The yellow fever mosquito that is responsible for epidemiological diseases such as Dengue, Chikungunya and Zika

Aggregation pheromone An odor that attracts members of the same species (conspecifics) to the same location for mate selection or defence against predators

Agrotis ipsilon (black cutworm moth) A long-lived migrant species of moth (Noctuidae), polyphagous, widespread, damaging particularly in the Northern hemisphere and known to postpone its activities linked to pheromone production and reproduction

Agrotis segetum (turnip moth) An owlet moth of the family Noctuidae, largely spread particularly in Europe, species of the same genus than *A. ipsilon* with whom interspecific hybrids can be obtained in laboratory conditions

Alarm pheromone A highly volatile odor pheromone chemical used to alert nestmates for danger (predator) and colony defense

Aldehyde oxidase A metabolic enzyme that catalyzes the oxidation of aldehydes into carboxylic acids

J.-F. Picimbon (ed.), *Olfactory Concepts of Insect Control - Alternative to Insecticides*, https://doi.org/10.1007/978-3-030-05060-3

Allelochemical (*allelo* = "another") A chemical produced by an organism that has an effect on individuals of another species when released (e.g. toxic chemicals released by the plants in response to herbivorous attacks)

Alpha-helix A basic structure in the protein characterized by a rod-like spatial configuration

Alternative splicing A regulated process of intron removal during gene expression that results in a single gene coding for multiple protein isoforms

Anopheles gambiae The primary mosquito vector for the transmission of Malaria

Anosmia Inability to sense an odor or a perfume scent

Ant An eusocial (from Greek Ɛŭ = "good") insect that lives in colonies (nests) similarly to bees and wasps with whom they share common ancestry (order Hymenoptera)

Antagonist An organism that competes with another (one species is negatively affected); A drug or chemical that binds to a receptor and blocks (or alters) the biological response by interfering with the interaction to the natural compound at the same receptor site

Antennal lobe The region of the insect brain which receives the input from the antennae

Antheraea polyphemus, A. pernyi A giant silkmoth (Saturniid) with large, double-combed male antennae, with one cm^2 outline area and 60,000 sensilla trichodea, each 300 μm long

Antimicrobial Peptide (host defense peptide) A 12–50 amino acids-long peptide with the potency to kill microbes and/or modulate the immune system as part of the innate immune response found among the whole class of life, including insects

Aphrodisiac An odor released by the male to facilitate its acceptance by the female as found in pyralid moths

Apis cerana The common Asiatic or Eastern honey bee

Apis mellifera The common European or Western honey bee

Apoptosis (from ancient Greek àπόπ Ţ= "falling off") The process of programmed cell death that occurs in all multicellular organisms

Arbovirus (arthropod-born virus) An informal name in modern medicine to refer to viruses that are transmitted by arthropod vectors of infectious diseases

Arthralgia (arthro = "joint", algos = "pain") A pain in one or more joints symptomatic of epidemiological diseases vehiculated by insects

Arthropod An invertebrate organism having an external skeleton (exoskeleton), a segmented body, and paired jointed appendages (insects, arachnids, myriapods and crustaceans)

Atmosphere (from Greek atmos = "vapour" and sphaira = "sphere") A layer of gases such as argon, carbon dioxide, nitrogen and oxygen surrounding Earth, held in place by the gravity of Earth and maintained if this gravity exerted by Earth is high and the global layer temperature is low enough, among others

Bacillus A genus of gram-positive aerobic, motile rod-shaped bacteria (firmicute, the most heat-resistant organism known on earth)

Bacillus thuringensis A soil-dwelling *Bacillus* bacterium naturally occurring in the gut of caterpillars and commonly used for insect pest control

Base pair mismatch A typo change in the genetic sequence that causes a point mutation

Bemisia tabaci The sweetpotato whitefly, principal threat to green vegetation worldwide

Beta sheet Another major type of conformation (formed by β-strands) observed in protein structures

Bimodality The simultaneous use of two distinct conditions, modalities or systems

Biocontrol The aim of controlling insect pest species using other insects or organisms

Biologically relevant odorant An odor molecule (or chemical signal) that can induce specific behavioral changes

Biopesticide A naturally occurring substance (or pesticide) from animals, plants, insects or bacteria or even a mineral that can affect the physiology and thereby the behavior of an insect pest species

Biosensor (biological sensor) A device, used for the analysis of a particular substrate; it combines a biological component (enzyme, antibody or nucleic acid) with a transducer that converts the recognition event (or molecular reaction) into a measurable signal

Biotransformation enzyme An enzyme that mediates a specific change of a drug or molecule within a given tissue of a living organism

Bitter taste sensation An acrid biting sensation in the gustatory modality that is associated to activation of bitter taste receptors

Bombykol (*E*,*Z*)-hexadecadien-1-ol, the first ever described sex pheromone, discovered by Butenandt et al. (1959), that is released by the female silkworm moth to attract specifically the male silkmoth

***Bombyx mori* L.** (in French *le ver à soie*) The silkworm of the mulberry tree, symbol of Asia and primary producer of silk and model organism in the study of genetics, neurobiology, olfaction and pheromone

Ca^{2+} channel An ion channel which has selective permeability to calcium (Ca^{2+}) ions

Calcium influx A massive entry or arrival of Ca^{2+} ions inside the cell

Calling The behavior associated to pheromone release; at a precise moment of the night, female moths immobilize on a vertical support such as the stem of a green plant and devaginate a (pheromone) gland located at the abdominal tip; this is accompanied by continuous vigourous wing fanning presumably to help disperse the odor

Calmodulin (calcium-modulated protein) A multifunctional intermediate Ca^{2+}-binding protein that mediates various metabolic processes in insect and other eukaryotic cells

Calyx (from Greek kálux = "husk or pod") A flattened cap of neuropiles in the insect brain where most sophisticated computations occur for signal recognition

cAMP (3′,5′-cyclic adenosine monophosphate) A second messenger important for signal transduction in many organisms, including insects

Capacitance The ability of the neural circuit to collect and store energy in the form of an electrical charge

Carbamate An organic (toxic) compound derived from carbamic acid (NH_2COOH)

Carbon The key ingredient for most life on Earth, the elemental composite of the cell

Carbon dioxide A colorless gas made of a carbon atom attached to two oxygen atoms (CO_2) that occurs naturally in earth's atmosphere and water resources since Precambrian period (about 600 Mya)

Carbon world An unstable ancient world that has influenced evolution and perhaps can be found in the modern time rich in carbon samples, molecules and emissions

Carboxylesterase (carboxyclic-ester hydrolase) An enzyme that utilizes two substrates (carboxylic ester and water) to release two products (alcohol and carboxylate)

Central olfactory pathways A combination of multiple interconnected olfactory structures in the insect brain that processes odor information and triggers specific odor-guided behavior

Chain shortening The process by which a long carbon fatty-acyl (lipid) chain precursor is subjected to selective two-carbon chain reduction to produce a specific sex pheromone

Chemical barrier A fatty acid, a protein, a secretion or another substance that helps defend the body against pathogens

Chemical defence A life history strategy of insects, plants and many other organisms to produce toxic or repellent molecules against predatory attacks; it also includes chemicals that reduce plant (or insect) digestibility to avoid consumption

Chemoreception The sensory modality tuned to volatile and non-volatile chemical stimuli molecules

Chemosensory organ An organ that is able to detect the presence of specific chemicals or relates to the perception of chemical substances – In mammals including human it includes the main olfactory epithelium (MOE) and the vomeronasal organ (VNO); in insects it includes the antennae, legs and proboscis, but not essentially the gut, the fat body, the dermis (immune organ) or the pheromone gland

Chemosensory Protein (CSP) A family of small soluble proteins (four-Cys) ubiquitously expressed throughout the whole insect body, also in arthropods and bacteria, highly abundant in chemosensory organs as well as in other tissues, tuned to fatty acids and xenobiotic chemicals for multiple functions including development, digestion, metabolism, pheromone production and immune defense

Chronobiology The study of the periodic (cyclic) phenomena, of the biological rythms and of the effects of time on living organisms

Cicadella (green leafhopper) A jumping insect pest known to consume sugar on leaves of trees and many various other plant cultures

Circadian clock (*circa diem* = "about a day") An internal clock whose biochemical, genetic and molecular components drive specific changes in the insect behavior depending on rythms with a period close to 24 h

Circadian clock gene A gene that encodes a protein involved in circadian clock oscillation

Circadian rhythm A 24 h cycle in a physiological process within a living organism such as an insect or a plant

Circadian rhythm of pheromone production/emission A 24 h cycle in the regulation of pheromone production and release in mating behaviors of insects (moths)

Cis-7-dodecenyl acetate (Z7-12:Ac) A crucial pheromone chemical for male response to female sexual odors in moths (*Agrotis* noctuids)

Cis-9-tetradecenyl acetate (Z9-14:Ac) A second crucial pheromone chemical for male response to female sexual odors in *Agrotis* noctuid moths

Cis-11-hexadecenyl acetate (Z11-16:Ac) A third crucial pheromone chemical for male response to female sexual blend of odors in *Agrotis* noctuid moths

Cockroach (*blatta* = "insect that shuns the light") A very ancient type of insect (320 Mya) closely associated to food residues and human habitats (since Antiquity); it can adapt to various kinds of external environments such as cold and heat, adopt a social organization, a kin recognition, a group or swarm behavior, a collective decision-making for food choice, and a very peculiar courtship ritual in which the female (*Periplaneta*) eventually climbs on the male's back to devour the abdominal tergal gland, site of production for the sexual pheromone

Cognate ligand A ligand that is strictly required for protein interaction and function

Coleopteran An insect or species that belongs to the order Coleoptera (beetles)

Consensus A motif of conserved amino acid residues in a protein gene family

Contact pheromone (cuticular pheromone) A non-volatile odor or pheromone detected by direct contact with chemoreceptors on the antennae or tarsi of insects and thereby closely related to social insect species such as ants and termites

Courtship ("*faire la cour*") An attempt or a specific behavior of the male to seduce the female in a purpose of mating for reproduction

Cricket (gryllid) A type of nocturnal insect known for the song of males in search for mates and for a sophisticated hearing tympanic system (eardrums) on the front legs

CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/Cas) A system or technology for gene/genome editing based on archaeal and bacterial prokaryotic defense mechanisms against foreign viral DNA contamination

Crop protection A field research in agronomy and agricultural sciences for sustained development and high-throughput production of food supply, transgenic plants and leguminous cultures resistant to insects

C-terminal pentapeptide motif The region of PBAN with pheromonotropic activity

Current clamp recording An electrophysiological method for measuring the voltage across a cell membrane at a fixed current across the membrane

Cuticle (exoskeleton) The outermost part (the external armur) of the insect body, also in all arthropod invertebrates, involved in many functions such as defence against toxic chemicals and prevention of water loss

Cycle The time necessary for a sequence of a recurring succession of biological events or phenomena such as those associated to diapause and reproduction to be completed

Cysteine ((R)-2-Amino-3-mercaptopropionic acid) The amino acid residue (Cys) that harbors a sulfur atom and helps build disulfide bridges in specific protein structures

Cytochrome P450 (CYP450) A superfamily of enzymes that use a variety of small and large molecules as substrates in various chemical reactions from the electron transfer chain and exogenous toxic chemical degradation

Cytoplasmic incompatibility A phenomenon caused by bacteria living in the cytoplasm of gamete cells that results in sperm and eggs being unable to lead to viable offspring

Damage The harm, injury, impairment, loss or destruction in biological function or economic value of a sensory cell or an agricultural parcel

Danaus plexippus (monarch butterfly) An iconic pollinator species known on the American continent for winter mass migration

DDT (dichloro-diphenyl-trichloro-ethane) An organochlorine (chlorinated hydrocarbon) insecticide molecule known to be associated with Alzheimer's disease

DEET (N,N-diethyl-meta-toluamide) A renown insect (mosquito and tick) repellent molecule with some known secondary toxic effects on human

Dengue hemorrhagic fever A severe outcome of dengue disease resulting in bleeding, low levels of blood platelets and blood plasma leakage

Dengue shock syndrome A severe outcome of dengue disease, where dangerously low blood pressure occurs

***De novo* pheromone biosynthesis** (*de novo* = "from a new") The particular biochemical pathways in which specific metabolites (pheromone products) are newly biosynthesized typically from acetyl coenzyme A in the (moth) pheromone gland

Desaturase (fatty acid desaturase) An enzyme that removes two hydrogen atoms from a fatty acid, *de novo* producing a specific carbon/carbon double bond pheromone molecule

Detoxification The process of removing exogenous foreign toxic (xenobiotic) substances from an organism, a tissue or a cell

Deutocerebrum (from greek deuteros = "second") A part of the insect brain with numerous glomeruli (ball-like structures) where the axons of antennal receptor neurons end and connect with interneurons and with neurons projecting to higher brain centers; within a glomerulus the receptor neurons of similar odorant specificity converge, there is e.g. one glomerulus for CO2 receptor neurons, pheromone receptor neurons converge in the macroglomerular complex

Development (simple or incomplete metamorphosis) The biological process that all insects must undergo from eggs to the adult stage and reproductive status

Diapause A physiological state dormancy; a delay in development in response to regularly and recurring periods of adverse environmental conditions

Dipteran An insect or species that belongs to the order Diptera (flies and mosquitoes)

Disparlure A specific noctuid sexual pheromone (2-methyl-7,8-epoxyoctadecane) released by female gypsy moths, *Lymantria dispar*

Disulfide bridge A linkage (or bridge) enrolling a disulfide (S-S) bond usually derived by the coupling of two thiol (R-SH) groups within the same protein and/or two different molecular complexes or protein units

Drosophila melanogaster The common fruit fly or vinegar fly, most widely used model organism for biological research in immunology, genetics, life history evolution, trait inheritance, microbial pathogenesis, neurophysiology, olfaction, vision and neurorobotics

Drosophila suzukii The spotted wing *Drosophila*, major fruit, grape, cherry and berry crop pest species worldwide; it is the rare fly that infests fruit and berry during the ripening stage, in contrast to most species of flies that infest only rotting fruit

Duplication (gene or chromosomal duplication) A major mechanism through which new genetic (DNA) material is generated during molecular (genome) evolution through unequal crossing-over (misalignment of chromosomes) and/or retrotransposition event

Dysgeusia The alteration in taste and recognition of gustatory molecules

Eciton hamatum A species of army ant (Dorylinae) known to prey on the larvae of other social insects such as wasps and ants of genera *Dolichoderus* and *Camponotus*

Ecodrug A drug, chemical, agent or reagent with eco-safe property (see ORSA), which needs to be considered for insect control and ecosystem preservation; the required alternative to insecticides and other environmental pollutants

Ecosphere An Earth closed ecological system; The part of the atmosphere in which it is expected to breathe naturally without aid, cure or protection

Electroantennogram (EAG) A recording from insect antennae with both electrodes located within the hemolymph (blood space) but at different regions on the antenna; the voltage changes observed upon odor stimulation reflect mixed responses of many receptor neurons, including temperature effects

Electroantennography An electrophysiological technique for measuring EAG, the average output (sum of responses of many olfactory neurons activated) of an insect antenna exposed to a given odor

Encephalitis An infectious disease in human characterized by a sudden onset inflammation of the brain or the brain tissue

Endectocide Insecticide applied to the host to kill an endo- or exoparasite

Entomopathogen A chemical drug or a bacterial organism that can cause disease specifically in insects

Entrainability The ability of oscillators (or clocks) to be synchronized with an external periodic signal such as seasonal variation and/or day length (photoperiodism)

Enzyme kinetics The study of the chemical reactions and reaction rates that are catalysed (governed) by specific enzymes

Euarthropoda The phylum of "true" arthropods (arachnids, crustaceans, insects and myriapods); their cuticle is periodically shed to allow for continued growth

Exon A part of a gene that will encode a part of the protein (block or motif) after introns have been removed by RNA splicing

Fairyfly (fairywasp) A family of almost invisible beautiful very tiny insects with a feathery appearance, the most primitive family within Chalcidoidea (100 Mya), which has a very short lifespan at the adult stage; females have the antennae tipped with club-like segments (clava), while the male antennae are filiform and look like a long soft cotton fiber thread

Fatty acyl reduction The chemical process involving the gain of electrons in a fatty acid to yield a fatty alcohol (via a fatty aldehyde intermediate)

Food choice An impact that food, plant or prey selection has on the environment, health and life of many organisms

Food trail pheromone An odor chemical that builds a narrow and precise route for the (insect) organism to reach specific food sources

Formica rufa The red wood (or horse) ant that sprays formic acid from their abdomen

Free-running period A period or rythm that is not adjusted to 24 h cycle nor to any other artificial photoperiodic cycle

Glutathione-S-Transferase (GST) (ligandins) A family of metabolic enzymes that catalyze the conjugation of the reduced form of glutathione (GSH) to foreign xenobiotic substances, participating thereby to cell or tissue detoxification

GABA (gamma-aminobutyric acid) A neurotransmitter that acts at inhibitory synapses by binding specific receptors in the membrane of both pre- and postsynaptic neuronal processes; it regulates brain and nerve cell (neuron) activity by decreasing the number of neurons firing in the insect (and human) brain

Genetic code Building blocks of life (Watson and Crick 1953); Genetic information in DNA conveyed solely by the linear sequences of four (nucleotide) bases (A, T, G and C) in a triplet codon alphabet that is used by living cells to translate gene/RNA into protein (most of all amino acids in the protein are specified by more than one codon or nucleotide base triplet in the DNA = degeneracy of the genetic code)

Genome A complete set of DNA (genes, exons and introns) that contains all the information necessary to build an organism and lead its activity through expression of a complete and specific repertoire of proteins

Glomerulus (*glomus* = "ball of yam") A globular structure or neural network of entwined vessels, fibers and nerve cells (neurons)

Glutamate An excitatory neurotransmitter in the (insect) brain essential for normal brain function, learning and memory

Glycine (aminoacetic acid) The simplest possible amino acid residue (Gly) that has a minimal side chain (one single hydrogen atom) and therefore can fit into any hydrophilic (attracted to water) or hydrophobic (not attracted, even repulsed by water) medium; it has a repeated role in the modulation of alpha-helical motifs in many various proteins

G-Protein Coupled Receptor (GPCR) A protein located in the cell membrane compartment (seven transmembrane domains) that binds extracellular substances and transmits specific signals though an intracellular relay molecule called G-protein (guanine nucleotide-binding protein)

G protein-coupled receptor kinase 2 (GPCRK2) A family of protein enzymes that regulate the activity of GPCRs by phosphorylation/dephosphorylation process

Guillain-Barre syndrome (GBS) A rare disorder caused by the immune system damaging the peripheral nervous system (= nerves outside the brain and spinal cord)

Gustation One of the five senses that belongs to the gustatory (taste) system

Haplotype A group of genes that are inherited together from a single parent

Heliothis virescens (tobacco budworm) A species of noctuid moths whose larvae are addicted to gluttony on cotton, pea, soybean and tobacco with extremely high resistance to a large panoply of insecticides

Hemocyte A cell from the hemolymph that plays a role in the immune system of insects (analogous to human phagocyte)

Hemolymph A transport fluid from the circulatory system that fills in the body cavity and all tissues in insects as well as in other arthropods (rather analogous to human lymph, not to human blood); it does not help carrying oxygen, it helps fighting infections and removal of waste toxic products

Histamine A biogenic amine inhibitory neurotransmitter in the insect brain

Honey bee (*Apis mellifera*) the most beneficial insect for human; building most intimate interactions with flowers, it provides human with honey, beeswax and crop pollination

Host plant odor A specific odor profile released by a plant most suitable for the moths or butterflies that need to lay eggs on it

Host preference The choice of an insect to find most suitable individual, organism, species, nest or plant for blood meal, food source, egg-laying and/or reproduction

Host selection The use of both olfactory and visual cues in (plant) host location

Hyalophora cecropia (cecropia moth) A giant silk moth with beautiful feathery antennae used to detect pheromonal odors from miles away. Also known for the discovery and extraction of juvenile hormone (1956) and as a symbol of North-American natural fauna

Hydrophobic semio-chemical A chemical signal used a mean of communication between organisms that can dissolve in the air, but not in the water

Hymenopteran An insect or species that belongs to the order Hymenoptera (ants, bees, sawflies and wasps)

IMD (immune deficiency) A key component of the immune response to infection specifically in the insect gut

Immunity The ability of an organism (including insects) to resist an infectious agent, a pathogen, a toxin or toxic xenobiotic substance by the action of the immune system

Inhibition of receptor neurons Nerve impulse firing possibly inhibited (i) by poisons affecting the nerve impulse generation (e.g. permethrin), (ii) by antagonistic ligands blocking odorant receptor molecules (e.g. presumably decanoyl-thio-1,1,1-trifluoropropanone selectively inhibiting pheromone-sensitive neurons of moth species), and (iii) by odorants that produce receptor potentials of opposite polarity thereby decreasing the spontaneous nerve impulse firing (e.g. linalool that inhibits some olfactory receptor neurons but excites others)

Inositol 1,4,5-triphosphate (IP3) (combined with diacylglycerol or DAG) A secondary intracellular messenger molecule used in sensory signal transduction and

lipid signalling that is known to diffuse through the cell to release intracellular calcium stocks

Insect (*insectum* = "with a divided body": head, thorax, abdomen) The largest group within Arthropods (a profusion of species); The most diverse kind of arthropod, characterized by a pair of antennae erected on the head, six legs and one or two pairs of wings at the adult stage- A panoply of developmental and reproductive variations- A set of sophisticated appendages or glands to make sounds or odors – A set of remarkable very sensitive and specialized organs of sensory perception- An example of parasitism or essential beneficial role- Their appearance and survival coincide with first Earth's terrestrial ecosystems (500 Mya)

Insect antennae Paired head appendages carrying numerous sense organs (sensilla) for detecting stimuli of various modalities: odorants, CO_2, taste compounds, mechanical stimuli (e.g. touch, vibration, sound), temperature

Insect behavior A very wide range of innate activities from pheromone communication to reproduction and migration, also including a whole panoply of diverse responses to environmental (toxic chemical) changes

Insect growth regulator A chemical substance that inhibits the life cycle of an insect

Insect pest An insect species that causes specific damages on crops or food supplies or poses a real threat to human health

Insertion mutation A type of base (or amino acid) mutation characterized by the insertion of one or few nucleotide base pairs to a DNA or RNA strand and/or the insertion of one or few amino acid residues (Glycine) to a protein motif or structure

Intron The silent (non-expressed) part of a gene, laying between two exons; it helps assemble exons but is removed from RNA after maturation before protein synthesis

Inversion mutation A type of base (or amino acid) mutation characterized by the removal of a length of DNA or a pair of amino acids which is then reinserted in the opposite direction in a protein motif or structure

Iodobenzene An organic compound with a benzene ring and one iodine atom

Ion channel A protein of the cell membrane serving as a gate for ion currents across the membrane; it may be opened upon specific (odor or neurotransmitter) ligand binding

Ionotropic receptor (ligand-gated ion channel) A family of ion-channel proteins located in the cell membrane which allow ions (Na^+, K^+, Ca^{2+} and/or Cl^- to enter the nerve cell in response to the selective binding of a chemical messenger neurotransmittor (or ligand)

Ipsdienol The aggregation pheromone ((4S)-2-methyl-6-methylideneocta-2,7-dien-4-ol) of bark beetles

Juvenile hormone (JH) A (main) hormone in insects, secreted by two tiny translucent endocrine glands near the brain (*corpora allata*), which play a crucial role in controlling most of the key processes in the insect physiology from development and molt to growth and reproduction through chemical communication, migration and oviposition

Juvenile hormone binding protein (JHBP) A protein that interacts with or helps the transport of JH in the hemolymph or in different compartments of the target cell to control specific gene expression

Kenyon cell An intrinsic nerve cell (or neuron) from the mushroom body of insects

Labial palp pit organ glomerulus The part of the insect brain tuned to CO_2 detection

Lamellocyte A large flat cell of the insect immune system that is known to function as a plasmatocyte (hemocyte)

Lateral horn (*lateral protocerebrum*) One of the two areas in the insect brain (the other area is the mushroom body) where projection neurons of the antennal lobe send their axons and specific odor information

Lepidopteran An insect or species that belongs to the order Lepidoptera (butterflies and moths)

Leucophaea maderae (Madeira cockroah) The first organism where an endogenous circadian clock was identified

Ligand-induced internalization An uptake of a material into a different compartment

Ligand-induced internalization of Ca^{++} into a receptor neuron A mechanism (desentization process) controlling odorant receptor signaling to ensure the appropriate cellular responses to a specific odor molecule

Linked gas chromatography-electrophysiology A technology that combines separation of pheromone volatile chemicals vaporized without decomposition (gas chromatography) and recordings from single olfactory neurons (electrophysiology) to screen for biological natural active novel compounds

Lipids A group of (oil, fat, wax and other ester) organic compounds strictly insoluble in water (highly hydrophobic); it is (with carbohydrates and proteins) the primary structural component of living cells

Local neuron (interneuron) A broad class of nerve cells that enable communication between sensory neurons and the central nervous system in the insect brain

Locomotor activity rhythm (*locō* = "from a place") A strong regular repeated pattern of movement from one place to another, largely under the control of a persistent endogenous timing mechanism of circadian frequency

Locust A solitary or gregarious insect (grasshopper) that can migrate in gigantic swarms and cause immense damages on cultures, vegetations and crops

Locusta migratoria The migratory locust that can change characteristics or traits (phenotype; from solitary to gregarious) in response to population density and build swarms of 40–80 millions individuals

Log_{10}-unit of stimulus intensity A step of factor ten in stimulus strength

Lymantria dispar (gypsy moth) The most destructive pest (Lymantriidae) of hardwood trees in US and North-America

Lymph emulsion A water-in-oil emulsion; a suspension of lipid droplets of oil in a water environment with which the oil will not mix

Maculopapular rash A type of rash characterized by a flat, red area on the skin that is covered with small confluent bumps

Mamestra brassicae (cabbage moth) An invasive noctuid species of moth known to feed (at the caterpillar stage) on many various fruits, vegetables and crops (cabbage, broccoli, Brussels sprouts, tobacco, tomato, sunflower, etc)

Management The process of dealing with or controlling insect pests

Manduca sexta (hawk moth; in French *le sphinx*) A species of moth (Sphingidae) that feeds on flowering plants (Solanaceae or nightshades) from agricultural crops, medicinals, spices, weeds and ornamentals, and a common model organism in odor neurobiology

Mating The action of pairing for intersexual interaction or reproduction

Microcephaly A medical condition present at birth or later during the first few years of life in which the brain does not develop properly resulting in an abnormally small head

Microfilaria An early stage in the life stage of parasitic nematodes (worms) that can be taken up from an individual (host) by blood-feeding insects and develop to infective larvae transmitted to a new host prone to cause epidemic diseases

Migration Seasonal flights or movements of insect species such as beetles, butterflies, dragonflies, locusts and moths (most damaging) in response to environmental changes

Molecular receptive range The agonist (excitatory) and antagonist (inhibitory) characteristics of an odorant receptor

Mosquito A long-legged buzzing dipteran fly with aquatic larvae and female that feeds on human blood transmitting a series of serious epidemiological infectious diseases (Chikungunya, Dengue, Malaria, Zika, etc)

Moth A crepuscular or nocturnal insect species with gluttonous herbivorous (phytophagous) larvae, females with pheromone gland at the abdominal tip and males with prominent hairlike or feathery antennae which flies at night to find the females that emit the odor over kilometers distance

Multiglomerular structure A (brain) structure that affects, contributes or pertains to multiple glomeruli

Musca domestica (house fly) The most common species found on cattle farms, a nuisance that can transport vector-mediated diseases; it is also a key element in ecological chain for breaking down and recycling organic matters

Mushroom body (*corpora pedunculata*) A pair of nervous structures in the insect brain known to play a key role in olfactory learning and odor memorization

Mutation A change, not necessarily an alteration, in the DNA, RNA or protein sequence that helps produce a new gene, RNA or protein isoform, prelude to new function in a given gene protein family in responses to specific external environmental changes

Myalgia A pain in one or more muscles

Mymar pulchellum A genus of fairyflies in Euathropoda Insecta Hymenoptera Mymaridae (only ten species described)

Mythimna separata The rice-ear cutting caterpillar; the major pest of maize in Asia

Negative staining The staining of the background used in transmission electron microscopy in order to increase contrast to the specimen

Nerve impulse (of sensory neuron) An action potential elicited (or suppressed) by the receptor potential reaching the impulse generator zone; this zone is thought to be located in the soma (cell body with nucleus) of the neuron, nerve impulse may also be spontaneously generated

Neuropile An area in the insect brain or any nervous system composed mainly of nerve fibers (only a few nerve cell bodies) that forms a synaptically very dense region

Niemann-Pick type C2 protein (NPC2) A small soluble β-stranded protein important for cholesterol, fatty acid and sphingolipid transport in the lysosome of animal cells and the sensory lymph of ant workers

Noxious compound detection The sensory perception of chemicals that are harmful, eventually destructive and difficult to control or eliminate (toxicants)

Noxious compound protection A mechanism in the insect defense system that allow them to cope with the toxic secondary compounds from the plant for specialization, selection and specific adapation to a potentially new habitat (host)

Nuptial gift A piece of food, twig of wood, tuft of grass or very precious bowl of silk that is given by the insect male to the female prior to mating

Octopamine The insect noradrenaline; it regulates aggression, behavioral development, reproduction, sleep, flight and odor memorization in various insect species, modulating specific neural signals in olfactory learning and memory as well as circadian rythms of sleep and activity for instance in honey bees, fruit flies and crepuscular moths

Odonatan An insect or species that belongs to the order Odonata (damselflies, dragonflies and Libellulidae)

Odor A scent, a stench, a bad or neutral smell that is caused by one or more in a bouquet airborne chemical volatiles all perceived by the sense of olfaction (i.e. the human nose or the insect antennae); it eventually refers to fragrance (a flower aroma, a perfume, a good positive enjoyable smell) for the positive aspect of life

Odor discrimination The perceptual ability (of the brain) to detect and describe differences between odors or perfume scents

Odor perception The brain's interpretation of the activation responses of many peripheral sensory neurons from the human nose or insect antennae which are differentially sensitive to a wide variety of molecules or chemical odorants

Odorant-binding protein (OBP) A small soluble α-helical protein that binds to odor molecule (odorant) at the periphery of olfactory receptors in the insect antennae

Odorant clearance The process of removing (eliminating, cleaning out, washing out etc) any residual odorant molecule from the human nose or the insect antennae

Odorant-Degrading Enzyme (ODE) An enzyme that mediates the metabolism of volatile signal molecules crucial to sustained sensitivity and specificity in the insect olfactory system

Odorant inactivation (odorant deactivation) A chemical alteration of odorant molecules by specific enzymes (ODEs) that stop them interacting with receptor molecules

Odorant Reception Suppressing Agent (ORSA) An airborne volatile or non-volatile synthetic odor pheromone chemical structural analog with a subtle modification in the native molecular stretch for the ability to block specifically the functional binding sites of target olfactory proteins and/or to counteract with specific odor receptor activation

Odorant receptor (OR) (olfactory receptor) A seven-(pass)-transmembrane domain protein expressed in the cell membrane of olfactory (receptor) neurons that need to be activated by specific odor molecules before the sense of smell

Olfaction The sense of smell; the primary sense tuned to odor detection and recognition; One of the most ancient and primordial modality to sense the environment

Olfactory co-receptor (ORCO) A co-expressed and co-localized olfactory receptor protein that complexes with odorant receptor to form an odorant-sensing unit

Olfactory receptor neuron (ORN) (olfactory sensory neuron, OSN) The cell that transduces chemical odor signals into electric neural messages that are sent out to the brain for odor sensing (ten million in human, thousands to ten thousands in insects)

Ophthalmotropic (from greek ophthalmos = "eye" and tropic = "turned towards") An insect species (moths or flies) that have developed feeding habits and mouth parts typically tuned to animal eye secretion

Optic lobe A structure or pair of structures (left and right) found in the microbrain of insects that integrate sensory information from the eyes and certain auditory stimuli

Organophosphate The common name for phosphate esters or esters of phosphoric acid; it includes DNA, RNA and ATP but also most common insecticide phosphorous chemical

Orthopteran An insect or species that belongs to the order Orthoptera (crickets, grasshoppers, katydids and locusts)

Ostrinia nubilalis (in French *la pyrale du maïs*) The European corn borer (*E* and Z strains); A grass moth (Crambidae) pest of grain, known for hairbrushes or hairpencils (aphrodisiac organs) in the middle and lower abdomen that the male opens out like a fan during courtship to facilitate its acceptance by the female

Oviposition The act or behavior related to lay eggs in insects

Palindrome A DNA or protein sequence that is spelled the same way forwards or backwards

Parasitoid An insect (usually a wasp) whose larvae feed and develop within or on the body of another insect species (usually a moth caterpillar): an example of endoparasitism (when the parasite lives inside the host organism)

Patch clamp recording A voltage or current clamp recording with the mouth of the recording electrode tightly sealed (GOhm seal) to a small patch (piece) of the neuron plasma membrane containing one or a few ion channels

Pathogen An agent such as a virus or a bacterial microorganism that can cause infectious disease

Pattern recognition receptor (PRR) A protein expressed by the (insect) innate immune system that plays a role as a host-sensor; it detects molecules specific to pathogens

PBAN agonist A peptide molecule that can bind to and activates a PBAN receptor to induce (or stimulate) a PBAN response

PBAN/pyrokinin family (FxPRL amides) A large family of neuropeptides (PBAN, diapause hormone, melanization and reddish coloration hormone-MRCH, myotropin, etc) that bear the same amidated C-terminal tail (FxPRL) and regulate multiple various physiological functions in insects (Lepidoptera), i.e. development, cuticular coloration, flight, mating, muscle contraction, pheromone production and wing tanning

PBAN receptor (PBANR) A G-protein coupled receptor with seven-(pass)-transmembrane domains which triggers a specific signal transduction in the female moth pheromone gland leading to pheromone production in response of PBAN activation

Pedunculus (Peduncle) A stemlike structure that collects nerve fibres and thereby connects different regions from the central nervous system of the insect brain

Peptidomimetic A subtly modified peptide chain that mimics the effect of the natural peptide or a system similar to peptides (poly-N-substituted glycines or peptoids and amyloid β, Aβ or Abeta peptides)

Period A gene that is expressed in a circadian pattern to associate specific behaviors with circadian rythms, the primary circadian pacemaker in the insect brain

Peripheral clock A functionaly autonomous local oscillator in circadian timing active not in the brain but in many peripheral organs or tissues such as the gut and antennae of the insect influenced by light, temperature, hormonal regulation and/or fasting-feeding cycle

Periplaneta americana (American cockroach) The largest pest species of common cockroach with ability of limb regeneration at the nymphal stage, a cosmopolitan plague that can live more than a year, reproduces over six hundred days and leads to more than ~150 progenies/a year

Perireceptor event The interaction between two or more molecular elements (ligand, transport protein, scavenger protein, enzymes) at the periphery of the receptor protein with central or pivotal function (i.e. odor receptor in the olfactory system)

Perireceptor event in insect olfaction The extracellular processing of the odor molecule before and after its interaction with the receptor protein, such as binding to soluble odorant binding protein, transport and degradation by odorant-degrading enzyme

Peritrophic matrix A semi-permeable envelope of chitin microfibriles that surrounds food metabolites in the insect midgut essential for digestion and infection by pathogens

Permian The geologic period of time and system which spans about 50 million years from the Carboniferous period (about 300 Mya) to the beginning of Triassic (about 250 Mya); it corresponds to the largest mass extinction of life recorded in the history of Earth (also called the Great Dying: 96% of species died out), the end of Paleozoic era

Pherokine A molecule related to both pheromone and immunological systems

Pheromone (from Greek phérein = "carry", and hormáo = "to set in rapid motion, stir up", "hormone") A secreted or excreted odor molecule, an odorant factor or chemical signal that triggers a specific behavioral response in individuals of the same species

Pheromone Biosynthesis Activating Neuropeptide (PBAN) A neuropetide (33 amino acids) with functional C-terminal FxPRL-NH_2 tail produced in the insect head (suboesophageal ganglion) secreted via *corpora cardiaca* (neurohemal organs of insects) and released into the hemolymph (and/or the ventral nerve cord) for the induction and stimulation of *de novo* pheromone biosynthesis in the lepidopteran female moth pheromone gland at some crucial time of the night

Pheromone blend A few or multiple pheromonal odors aimed at combining different molecules into a species-specific uniform whole odorant signal

Pheromone degrading enzyme (PDE) An enzyme that specifically mediates pheromone degradation (catabolism) and/or the conversion of pheromone molecules into inactive (or less active) forms

Pheromone gland A primary source and reservoir for sequestrating *de novo* biosynthesized chemical compounds with pheromone function (e.g. the sex pheromone gland of female moths); it is usually covered by pines on the gland surface to facilitate pheromone emission and/or odor release

Pheromonogenesis The genesis of *de novo* (sex) pheromone chemicals via multiple key biosynthetic enzymes from the uptake of fatty acid, lipid or thioester precursor molecules to the final product of specific pheromone biosynthetic pathway

Pheromonostasis A mechanism or a peptide molecule mediating arrest or suppression of pheromone production in the sex pheromone gland; it naturally occurs in female moths after mating thanks to a number of humoral (male factors, sex peptides) and neural cues, it can also be induced by a family of biosynthetic sex peptide analogs inhibitors of sex pheromone production in selected insect pest species

Phospholipid A large biological polymer of the lipid family with hydrophobic "legs" (fatty acid) and hydrophilic "head" (phosphate) that plays a crucial role in the formation of cell membranes and all membranes surrounding organelles (= cell organs, differentiated structures within a cell that performs a specific function, e.g. mitochondria from the insect cell)

Phosphorylation The reversible process of attaching a phosphate group to a molecule (mainly on Serine, Threonine or Tyrosine amino acid residue) to help lead a protein to trigger a specific physiological mechanism (opposite: dephosphorylation); it is certainly one of the most important post-translational modification in various protein structures, including enzymes and receptors

Photoperiod The lenght of day or night in a cycle of time (24 h)

Photoperiodic clock An endogenous (internal) clock or timekeeping network that allows insects as well as many various organisms to align a specific physiological system with a changing external environment in order to perform most adapted biologically relevant important behavior

Photoperiodic response A functional physiological and/or behavioral change in response to a change in the length of day and night

Photoperiodism The physiological reaction of insects (and plants) to a photoperiod

Physical barrier An environmental, induced or natural condition that interferes in communication or interaction between two cells, individuals, organisms or species

Physiology The discipline of biology concerned with the functioning of living organisms

Pit organ A temperature- infrared- CO_2- and odor-sensitive organ on the antennae, or antenniform legs of insects (beetles, hymenoptera, moths), the small Haller's organ on the forelegs of ticks and varroa used to detect heat and pheromone chemical odors released by host (honey bee); it is formed by a ring-shaped cuticular ridge surrounding a pit (a hollow or indentation in the leg surface) containing five or six raised pore openings within each two to five sensilla are exposed

Plant-herbivore insect interaction A range of adaptations evolved by plants and insects for co-evolution: the responses of the plant to herbivore insect attack, the responses of the insect to plant defense, host-plant resistance, insect resistance, survival dynamics

Plant semiochemical (from Greek semeion = "signal") A chemical substance released by plants to defend themselves against herbivore insect attack by repeling the assaillant and/or by attracting natural ennemies (predators) of the herbivore (tritrophic interactions)

Plasmodium falciparum A unicellular protozoan parasite transmitted by *Anopheles* mosquitoes that is the main cause of malaria (anemia) disease in humans

Poison avoidance The act of avoiding (keeping away) from toxic chemical element possibly ingested (by insects) though food and nutrients

Poisson statistics Statistics of random events as e.g. arrival of single stimulus molecules on olfactory sensilla at a weak stimulus concentration

Proboscis The insect tongue (the sucking organ of a bee, a butterfly, a fruitfly or an hawk moth); an appendage, elongation or extension at the front of the insect mouth whose vital function remains elusive in most adult moths as most adult moths do not feed and do not suck nectar: proboscis should be absent when superfluous

Projection neuron An afferent (arriving to the brain) or efferent (exiting the brain) axonal projection fiber nervous cell uniting the insect brain with lower parts, peripheral nervous system, suboesophageal ganglion (SOG) and other ganglia of the ventral nerve cord that innervates (for instance) the pheromone gland in moths

Proline (pyrrolidine-2-carboylic acid) The only amino (imino) acid residue (Pro) with a pyrrolidine (or tetrahydropyrole) and amine function for side chain, which confers an exceptional conformational rigidity in protein structure; it is usually found at the beginning of alpha-helices and in the edge strands of beta-sheets: polyproline motifs are essential for protein phosphorylation, protein assembly and signalling

Protein The core of life in cells (with lipids and other molecules); a short or very elongated soluble or trans-membrane macromolecules consisting of one or multiple chains of amino acid residues (such as Cysteine, Glycine and Proline) that

combines to build the (primary) structure dictacted by the nucleotide sequence of the corresponding gene on the basis of the genetic code (Watson and Crick); specific amino acid motifs can adopt different types of (secondary) structures (alpha-helix, beta-sheet and beta-turn) and foldings (tertiary structure) to underlie specific cell functions in adhesion, cycle, development, division, growth, shape, catabolism, metabolism, transport, regulation, signalling and immunological responses; to fullfill these tasks in multiple systems, proteins are often subjected to post-translational modifications (see phosphorylation) and it is said that the protein can even be subjected to specific (Cys, Gly or Pro) insertion mutation or inversion to acquire multi-function

Protein structure model (homology-modelling) An inference of protein's tertiary (3D) structure (prediction of alpha-helices and variations) from its amino acid sequence based on the known 3D crystal structure of a homologous protein used as reference or template

Protein variant (protein isoform) A representation of changes (mutations) in the amino acid sequence encoded by a specific DNA sequence (gene) in the genome; A new protein sequence in the repertoire of highly similar proteins that originate from the same gene but differ by one or a few amino acid replacements, the simplest variant (isoform or mutant) being the protein in which only one amino acid was subtly replaced by another to induce a new protein function

Protocerebrum The region of the insect brain innervating the compound eyes; it includes important higher centers like the mushroom bodies and the central body

Protozoan A rather informal term to refer to unicellular eukaryotic organism (or protist); a main class of parasites that cause infectious disease (Malaria) in human

Pyrethroid An organic insecticide compound similar to the natural pyrethrin molecule from pyrethrums flowers (*Chrysanthemum cinerariaefolium*)

Receptor potential A change of electrical voltage indicating the excitation of a sensory neuron, the stimulus-induced change of neuronal membrane conductance; it may be recorded extracellularly using capillary electrodes, with the "indifferent" reference electrode in contact to the hemolymph or blood space, and the "recording" electrode positioned near to the apical portion of a sensory neuron. The polarity of the receptor potential is negative or positive if the neuronal membrane conductance is increased or decreased upon stimulation

Receptor potential/current, elementary (ERP/ERC) An elementary small transient voltage/current wave ("bump" or group of "bumps") elicited by a single odorant molecule or (infrequently) spontaneously

Repellent An odorant chemical molecule that can elicit an aversive or repulsive behaviour specifically in some insect pests or predators

Reproduction The biological process by which a new individual organism (descendant or offspring) is produced from a « mother » and a « father » parent; one of the most important concept in biology in which an organism is born and tends to make a copy or a likeness of itself to sustain and give a chance for a species, a genus, a family or an order to survive and/or have a continued existence during the process of evolution

Retrotransposon (transposon via RNA intermediate) A genetic element that can copy and paste itself at many different locations in a genome eventually inducing mutations by inserting near or within a particular gene sequence

Rhizophere The region of soil where interactions between plant roots and associated bacterial microorganisms take place

Rickettsia A genus of bacteria of the tribe Rickettsiae; A small, nonmotile, non-spore forming, highly pleomorphic (occurring in many various distinct forms) rod-shaped to coccoid bacterial organism that lives in the body of lice or ticks and is responsible for Mediterranean spotted fever in humans

Riptortus pedestris An alydid hemipteran insect species (bean bug) extremely polyphagous; one of the major pests on leguminous crops (soybean), whose diapause is tightly regulated by circadian cycle and endogenous clock genes

RNA (ribonucleic acid) A polymeric single-stranded molecule that conveys the information from DNA to protein and therefore represents one essential core for gene expression and cell function; the origin of life: the components (chains of nucleotides, ribose and phosphate) built on crust in space and assembled on Earth

RNA-DNA difference (RDD) (mismatch or mutation) A site of base replacement or switch between DNA and RNA sequences during transcription (= copy of DNA to RNA) or following specific RNA editing by ADAR enzymes

RNA editing The guided post-transcriptional (= after copy of DNA to RNA) subtle modification of RNA sequence from the genomic DNA sequence that can lead to high number of protein variants and thereby multifunction from a single gene

RNA interference (RNAi) A mediated knockdown process in which specific RNA molecules inhibit the expression or translation of a specific gene resulting in the absence of a target protein in a given cell, tissue or organism

Scavenger An insect (fly or wasp) or a protein that feeds on or interacts with the residual matter, keeping a dust-free environment (or fluid) by specific nature recycling processes

Schistocerca americana (American bird grasshopper) The main pest for (palm) trees and lemon crops in Florida, also know for a specific family of fatty acids (caeliferins) from the grasshopper regurgitant that induces the plant to release allelochemicals

Schistocerca gregaria (gregarious desert locust) One of the most dangerous and threatening insect species for humans; it can build swarm of 50–100 billion individuals and can eat up one/tenth of human agricultural production and food supply in three main parts of the world (Africa, Middle-East and Asia)

Second messenger A molecule inside the cell that transmits a specific signal from a transmembrane receptor to an intracellular target (the first messenger being the hormone or the odor chemical that conveys the signal to the cell)

Selectivity The quality of the insect olfactory system of discriminating, selecting and carefully choosing an odor as the most suitable

Seminal fluid (semen) A fluid that is produced by the male reproductory tract secretory tissues (accessory glands, seminal vesicles, ejeculatory duct and testis) and that contains sperm cells (= spermatozoa) and proteins that are transferred

to females with sperm during mating, resulting in specific changes in female behavior and physiology (pheromone inhibition, rejection of male, facilitation of feeding, ovulation and ovogenesis/egg production)

Sensillum (plural: sensilla) A small epithelial sensory unit including a cuticular structure (hair, plate) supplied with (often three) auxiliary cells, and innervated by one or several receptor neurons; in hairlike sensilla (hair length 10–500 μm) the apical neuronal processes (dendrites) may extend throughout the hair shaft, the axons of the receptor neurons conduct the nerve impulses to the central nervous system in the insect brain

Sensillum lymph The aqueous fluid that baths the dendrites of olfactory neurons with pheromone solubilization/emulsification by binding to proteins, pheromone transport and degradation (see perireceptor events)

Sensillum type, gustatory and mechanoreceptive S. chaeticum (bristle, innervated by several taste neurons and often one mechanoreceptor neuron ending at the sensillum base)

Sensillum types, olfactory S. trichodeum (very long hair), s. basiconicum (short hair), s. coeloconicum (very short hair, sitting in a pit), s. placodeum (pore plate, in bees and beetles), *s. ampullaceum* (deeply hidden hair, found in ants for CO_2 detection)

Sensory adaptation A reduction in the responsiveness due to preceding stimulation, observed in responses of sensory receptor neurons and in behavioral responses

Sensory transduction The sum of processes in which a signal chemical (odorant, tastant) induces a receptor potential and impulse firing of a receptor neuron; this may happen via direct gating of ion channels or include a cascade (a series of) molecular events such as protein phosphorylation, second messenger formation, and release of intracellular Ca^{2+}

Serotonin (5-hydroxytryptamine or *5HT*) A monoamine neurotransmitter that acts also as a systemic hormone in insects where it regulates circadian rythms, gut motility, tissue secretion, development, growth, locomotion, flight, learning and memory

Serotype A group of intimately related microorganisms distinguished by a common set of antigens or the set of antigens characteristic of this group

Sex pheromone A long-range highly volatile natural odorant pheromone chemical usually released by the female from a peculiar organ such as the sex pheromone gland of female moths to attract a conspecific male on a precise location, cocoon or plant site prelude to mating and reproduction

Sex pheromone gland A layer of glandular epithelial cells sandwiched between ovipositor and sclerotized cuticle at the tip of the female abdomen; A very active site for lipid and pheromone droplets, specifically devaginated (in nocturnal species of moths) during calling behavior at crucial moment of the night for release of sexual odor volatiles

Species similarity and difference A fundamental resemblance or common point, an homology (a shared ancestry) and/or an analogy (an apparent resemblance of

structures that clearly have different origins but similar function) and dissimilarity (or dissemblance) between different species

Specificity, neuronal The pattern of stimulatory chemicals producing excitatory and inhibitory responses of a receptor neuron; pheromone receptor neurons may have an extremely high specificity in responding >100-fold less sensitive if the pheromone structure is minimally changed, other neurons may respond to a number of chemicals in various proportions

Sphingolipid A lipid with sphingosine (a molecular structure shape as enigmatic as a Sphynx) that accumulates in tissues as diverse as the liver and the brain to regulate diverse cell functions in response to cellular stress (mainly oxidative stress)

Sphinx ligustri (privet hawk moth) The sphinx of the Paleartic zone (Europe and Asia)

Spodoptera frugiperda (*frugiperda* = "lost fruit") The fall armyworm, a severe case study of cannibalism and herbivory in noctuid moths

Spodoptera littoralis (African or Egyptian cotton leafworm) The Mediterranean brocade labeled as quarantine pest (40 different plants and at least 87 different plant species) that feed on young leaves, young shoots, stem, pod, bud and fruit throughout the whole world

Stem cell An adult or embryonic cell that can differentiate into another type of cell and function to produce even more of these new cells and functions

Streptomyces The largest genus of *Actinobacteria* (about 500 Mya); the most adapted organism to the utilization of plant and soil residuum in all various environments

Structure activity relationship The relationship between a chemical (or drug ligand) and/or a 3D structure of a protein molecule and their biological activity

Suboesophageal ganglion (SOG) A part of the ventral nerve cord below the oesophagus inside the head in insects (and arthropods) connected to the brain and to the first thoracic ganglion that controls the mouthparts and salivary glands but also produces neuropeptides (e.g. PBAN) that will stimulate the pheromone gland at the abdominal tip

Sugar taste inhibition The loss of sweet taste perception as a result of the alteration in the activation of sweet taste receptors and/or a neurobiological disturbance in the insect brain or ventral nerve cord

Surface tension The attractive force exerted upon the surface molecules of a liquid by the molecules beneath; it tends to draw the surface molecules into the distinct mass of the liquid and makes the liquid such as water assume a shape with the least surface area (e.g. water or lymphatic surface in contact with air)

Surfactant A solute or substance which tends to reduce the surface tension of a liquid in which it is dissolved

Swarming A collective behavior displayed by insects of the same species (locusts, butterflies, moths, beetles, flies, mosquitoes, aphids, whiteflies, wasps, termites, flying ants and most other winged insects) to aggregate together, move in large numbers and migrate towards specific geographical locations to reproduce or continue development

Synapse (from Greek synapsis = "conjuction") A structure or intercellular space where a neuron (or nerve cell) connects another neuron or a target cell and propagates a specific chemo-electrical signal

Taste (gustation) The primary sense used by human, animals and insects to distinguish one potential food source from another

Taste sensillum A bristle-like sensillum (chaeticum) on the insect maxillary palp (mouthpart) or insect antenna responsible for sweet sugar detection

Temperature compensation A phenomenon in which the output of the endogenous clock system remains nearly constant with fluctuations in external temperature

TEP protein (thioester-containing protein) An antimicrobial protein from the insect immune system that uses a specific thioester motif to damage the cell membrane at the surface of the invading infectious pathogen

Termite Eusocial insect that evolved from an ancestor of cockroaches (about 300 Mya) and entirely tuned to digestion of cellulose that the wood is made of

Tip recording The recording capillary electrode is slipped over the tip of a hair-like sensillum in order to record receptor potentials and nerve impulses from the sensillar neurons; in olfactory sensilla the hair tip may be opened for improving the electrical contact to the neuronal dendrites inside the hair shaft, taste sensilla (sensilla chaetica) have a terminal opening that receives tastants and also allows electrical contact to gustatory neurons and to a mechanoreceptive neuron

Toll receptor An immune receptor in the membrane of sentinel cells (macrophages) from the insect adaptative immune system that can recognize molecules that are broadly shared by pathogenic microbes (sense internal danger signals) and trigger many various responses of the insect defence system, including antimicrobial peptides, proinflammatory cytokines and chemokines

Transcript (mRNA) A single-stranded mRNA product synthesized by transcription of a genomic DNA sequence, eventually subjected to editing and processed for translation (protein synthesis); multiple transcripts or mRNA sequences do no mean necessarily multiple genes, a gene can lead to multiple transcripts and therefore to multiple proteins

Transcription The process in which the genetic information from DNA is transcribed into RNA by a specific enzyme called RNA polymerase

Transepithelial recording A tip recording implemented if the indifferent electrode is located basally from the epithelium; in cases of high electrical resistance across the epithelium (e.g. 200 MOhm), loose patch clamp conditions exist where the neuronal dendrites represent the patch of cell membrane

Translation The process in which the genetic information from RNA is translated into specific amino acid chain, protein or polypeptide before further editing and/or folding for the final protein product to perform specific function within the cell, tissue or organism

Truncation A mutation which induces premature stop codon thereby producing a shorted protein with a truncated (aborted) tail

Type I pheromone A major group of moth sex pheromones composed of a 12–18 carbons-long fatty acid chain (with one, two or three double bounds and trans (*E*) or cis (*Z*) isomers) connected to an oxygenated functional group (acetate, alcohol

or aldehyde) as the only polar and therefore hydrophilic (water loving) portion of the molecule

UDP-Glycosyltransferase An enzyme that catalyzes the addition of a glycosyl group from a uracyl-diphosphate (UDP) sugar molecule to a small hydrophobic (water hating) fatty acid chain

Varroa destructor An external parasitic mite that can only lives attached to the body of honey bees, spreading varroosis disease and deformed wing virus in the colony or hive

Vector An agent or organism (invertebrate arthropod such as insect) that carries and transmits an infectious pathogen reponsible for epidemic disease into another living organism such as human

Visual pigment (rhodopsin) A G-protein coupled receptor molecule consisting of a protein (opsin) and a vitamin A-derived chromophore (11-cis retinal) that plays a key role in image formation in visual receptor neurons in both *Drosophila* and human eyes

Volatile organic compound (VOC) An organic chemical that has a high vapor pressure and low boiling point at normal temperature, which causes the chemical molecule to easily change to gas from the liquid or solid site of production and evaporate into the surrounding air (volatility); it probably includes most naturally-occurring odorants, most scents, odors or perfumes that play a key role in communication between plants and between plants and other organisms (including insects); for instance, specific subset of VOCs or green leaf volatiles that are released by damaged plants upon herbivore attacks in order to attract the herbivore natural enemy (predator) while alerting the other plants about the herbivore attacks

Voltage clamp An electrophysiological method for measuring the current across a cell membrane at a fixed voltage across the membrane

Wolbachia The most common inherited parasitic endosymbiotic bacterial species naturally present in more than 60% of insect species (including wasps and mosquitoes); the *Wolbachia*-mediated infection can result in cytoplasmic incompatibility and embryonic mortality in specific insect pest species

Xenobiotic A drug chemical substance that is foreign (exterior) to a biological system

Xenobiotic metabolizing enzyme A family of enzymes that modulate cellular interaction with environmental xenobiotic chemicals (insecticides or toxic pollulants) by degradation or modification (recycling) of the xenobiotic chemical structure

Zeitgeber An external environmental factor (e.g length of daylight or temperature) that helps setting (or re-setting) the rythm of a biological clock