Tongxian Liu
Le Kang
Editors

Recent Advances in Entomological Research

From Molecular Biology to Pest Management

高等教育出版社
HIGHER EDUCATION PRESS

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With 87 figures, 3 of them in color

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Preface

Insects represent the most diverse group of species at planet earth, accounting for over 50% of known organisms. Their close interaction with human and other life forms has significant impacts on human health, environments, agriculture, biosafety, etc. Thus, entomology has been a hot research topic for worldwide scientists for long time. The development of modern biology such as molecular biology, cell biology, genetics, integrates new elements and concepts into the classical entomology. Now, over ten insect genomes have been sequenced. These data, plus the novel tools and thoughts, provide tremendous amounts of information for entomological researchers to deeply and systematically study insects.

We, as entomologists, were fascinated, and then were inspired to edit a book to present such rapid advances and progresses in entomological research. This motivation was realized as a result of our opportunity in interacting with numerous entomologists during academic research. We invited more than forty scientists with research specialties ranging from molecular biology to pest management to contribute chapters with a most comprehensive overview to date to include most, if not all, recent advances in their field of specialties.

This book contains 25 chapters, ranging from molecular biology to applied pest management, authored by 49 scientists. The first section, Insect-Plant Interactions, include five chapters, covering deciphering the plant-insect phenotypic arms race, inducible plant defense against insect herbivores, host marking and host discrimination in phytophagous insects, and plant's defense modulated by minerals.

The second section, Molecular Biology, Physiology, Behavior and Ecology, comprises seven chapters, including recent advances in virus infection in honey bee, biological function of insect yellow gene family, the function of bursicon, a neuropeptide hormone, chemical ecology of bark beetle, inforchemical tritrophical interactions in soybean aphids-host plants-natural enemies, the response of insects to global warming, and the biology and reproductive strategies of the subterranean termites.

The third section, Insect Toxicology and Insecticide Resistance Management, consists of seven chapters, including the roles of P450s in insecticide resistance and interactions with bioactive agents, metamorphosis of innate insect resistance in host plants research, new discoveries in genetically modified crops and natural enemies, and the molecular mechanism of insecticide resistance in mosquitoes and other insect pests of field crops.

The fourth section, Emerging Pest Management Strategies and Technologies, contains six chapters with a broad range from RNAi technologies, anti-tick vaccine, veterinary pests, biological and integrated management strategies of various field crops, invasive imported red fire ants, urban pest management, and an emerging area of entomological science that utilizes lignocellulose-feeding insects for viable biofuels.

We think that each chapter is sufficiently thought-provoking that it is expected to find its way onto the bookshelves of scientists, post-graduate students and advanced undergraduate students who are interested in insect molecular biology, insect-plant interactions, insecticide toxicology and resistance management, integrated pest management, and agriculture and urban entomology.

We would like to acknowledge the numerous referees that read and commented critically on each chapter. They are acknowledged in each chapter. Also, we want to acknowledge Miss. Dan Yu of Institute of Zoology, Chinese Academy of Sciences, Miss. Chao Pan of Higher Education Press, and people in Springer for their great assistance during the publication process.

Tongxian Liu
Le Kang
December 24, 2010

Contents

Section 1: Insect-Plant Interactions

1. Deciphering the Plant-Insect Phenotypic Arms Race ····· *Xianchun Li, Xinzhi Ni* 3
2. Insect Herbivory-Inducible Proteins Confer Post-Ingestive Plant Defenses ····· ····· *Keyan Zhu-Salzman, Tongxian Liu* 34
3. Inducible Direct Defense of Plants Against Insects ····· ····· *Mingshun Chen, Junxiang Wu, Guohui Zhang* 49
4. Host Marking and Host Discrimination in Phytophagous Insects ····· ····· *Siwei Liu, Baige Zhao, Edmond Bonjour* 73
5. Nitrogen Modulation on Plant Direct and Indirect Defenses ····· ····· *Yigen Chen, Xinzhi Ni* 86

Section 2: Molecular Biology, Physiology, Behavior and Ecology

6. Viruses and Viral Diseases of the Honey Bee, *Apis mellifera* ····· *Yanping Chen* 105
7. Biological Function of Insect Yellow Gene Family ····· ····· *Jianyong Li, Bruce M. Christensen* 121
8. Bursicon, a Neuropeptide Hormone That Controls Cuticle Tanning and Beyond ··· ····· *Qisheng Song, Shiheng An* 132
9. Chemical Ecology of Bark Beetles in Regard to Search and Selection of Host Trees ····· *John A. Byers, Qinghe Zhang* 150
10. Infochemical-tritrophic Interactions of Soybean Aphids-host Plants-natural Enemies and Their Practical Applications in Pest Management ····· ····· *Junwei J. Zhu* 191
11. The Responses of Insects to Global Warming ····· ····· *Kun Guo, Osbert Jianxin Sun, Le Kang* 201
12. Biology and Reproductive Strategies in the Subterranean Termites (Isoptera: Rhinotermitidae) ····· *Xingping Hu* 213

Section 3: Insect Toxicology and Insecticide Resistance Management

13. P450–mediated Insecticide Detoxification and Its Implication in Insecticide Efficacy ····· *Zhimou Wen, Xing Zhang, Yalin Zhang* 229
14. House Fly Ctyochrome P450s: Their Role in Insecticide Resistance and Strategies in the Isolation and Characterization ····· *Nannan Liu, Fang Zhu* 246

15. Metamorphosis of Cisgenic Insect Resistance Research in the Transgenic Crop Era ····· *Xinzhi Ni, Xianchun Li, Yigen Chen, Fuzhen Guo, Jinian Feng, Huiyan Zhao* 258
16. Time for a New Look at the Relationship Between Bt Plants and Insect Natural Enemies ····· *Mao Chen, Anthony M. Shelton* 280
17. The Development of Pyrethroid Resistance in the Mosquito *Culex quinquefasciatus* ····· *Qiang Xu, Nannan Liu* 295
18. Resistance to Transgenic *Bacillus thuringiensis* Crops in Target Insect Pests: Current Status and Prospect ····· *Fangneng Huang* 306
19. Potential Use of Proteinase Inhibitors, Avidin, and other Bio-reagents for Synergizing Bt Performance and Delaying Resistance Development to Bt ····· ····· *Yucheng Zhu, Mingshun Chen, Craig A. Abel* 330

Section 4: Emerging Pest Management Strategies and Technologies

20. Advances and Prospects of RNAi Technologies in Insect Pest Management ····· ····· *Xin Zhang, Jianzhen Zhang, Kunyan Zhu* 347
21. Anti-tick Vaccine Development: Status and Perspectives ····· ····· *Quentin Q. Fang, Oscar J. Pung* 359
22. Hymenopteran Parasitoids and Their Role in Biological Control of Vegetable *Liriomyza* Leafminers ····· ····· *Tongxian Liu, Le Kang, Zhongren Lei, Ricardo Hernandez* 376
23. Development of an Integrated Greenhouse Whitefly Management Program on Strawberries ····· *Jianlong Bi* 404
24. Advances in Research on the Venom Chemistry of Imported Fire Ants ····· ····· *Jian Chen, Hanwu Shang* 417
25. Utilization of Lignocellulose-feeding Insects for Viable Biofuels: an Emerging and Promising Area of Entomological Science ····· *Jianzhong Sun, Xuguo Joe Zhou* 434

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Section 1: Insect-Plant Interactions

CHAPTER 1

Deciphering the Plant-Insect Phenotypic Arms Race

Xianchun Li and Xinzhi Ni

Abstract Plants and herbivorous insects interact with each other on three different time scales. On the ecological time scale, the interacting species, both plants and insects, exhibit a back-and-forth attack-defense-counterdefense cycle, resulting in a phenotypic arms race. Such short-term changes in defenses and counterdefenses of individual plants and insects are mediated by reciprocal elicitation and regulation of gene expressions. All reciprocal regulation of gene expression, in turn, are stimulated by chemical or physical signals, from the environment, the organism itself, or the interaction partner. All signals, no matter internal or external, must be received and processed at the level of individual cells. A number of signals that trigger the reciprocal regulation of plant defense or insect counterdefense genes have been characterized. A growing number of microarray studies have been conducted to define the plant defense and insect counterdefense transcriptomes, i.e., genes whose transcription rate is altered by defense-counterdefense interactions. In this chapter, we reviewed the reciprocal signaling and transcriptome dynamic that underlie the plant-insect phenotypic arms race.

Keywords herbivore, plant-insect interaction, defense, counterdefense, macroevolutionary time scale, microevolutionary time scale, reciprocal signaling, transcriptome dynamic

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1.1 Introduction

Plants and insects have interacted with each other for millions of years (Gatehouse 2002). The interactions between the two taxa can be mutually beneficial (known as mutualism), with insects pollinating (e.g. honeybee) or protecting plants (e.g. *Pseudomyrmex* ants) from herbivores and plants in return providing foods and/or shelter for insects. The majority of interactions between the two groups, however, are antagonistic, involving insect herbivory of plants and plant defense against the herbivorous insects. Given its importance in ecology, evolutionary biology, and agriculture, the antagonistic plant-insect interaction has been investigated at different biological organization levels, from species (i.e. genomic arms race) to population (genotypic arms race) and individual (phenotypic arms race), and on different time scales, from macro-evolution (diversification of plant and insect species) to micro-evolutionary (evolution of novel plant defense and insect counterdefense gene alleles) and ecological time scale (induction of plant defense and insect counterdefense genes). This chapter presents the current understanding of the phenotypic arms race between the interacting plant and insect individuals at the ecological time scale, with emphasis on the plant-insect signaling interactions and the plant defense and insect counterdefense transcriptomes and proteomes.

1.2 Plant-insect phenotypic arms race

Plant-insect interactions have been studied at both the evolutionary and ecological time scales. Evolutionarily, the rapid diversification of plant-insect interactions may have been a consequence of reciprocal selection pressures whereby plants evolve biosynthetically novel defensive compounds and proteins, and insects (and other herbivores) overcome erstwhile toxins with novel detoxification pathways (Engler et al. 2000; Zangerl & Berenbaum 2003). As sedentary organisms that form the base of most food webs, plants are subject to intense selection pressure from herbivores, and have few options other than chemical or morphological defense for reducing the impact of herbivores (Berenbaum 1995).

Ecologically, the interacting species, both plants and insects, exhibit a back-and-forth attack-defense- counterdefense cycle (Agrawal 2001). The initial herbivore feeding damage provides plants with mechanical signals (wounding) and chemical/enzymatic signals such as volicitin (Weissbecker et al. 1999; Frey et al. 2000), similar fatty acid-amino acid conjugates (FAC) (Halitschke et al. 2001; Tumlinson & Lait 2005), bruchins (Doss et al. 2000), inceptin (Schmelz et al. 2006, 2007) and glucose oxidase (GOX) (Orozco-Cardenas et al. 2001; Musser et al. 2002, 2005) present in the oral secretions (OS) or oviposition fluids (OF) of insects (Fig. 1.1). These signals in turn lead to immediate synthesis and accumulation of plant defense-signaling chemicals including jasmonic acid (JA), salicylic acid (SA), and/or ethylene (ET) (Mello & Silva-Filho 2002; Kessler &

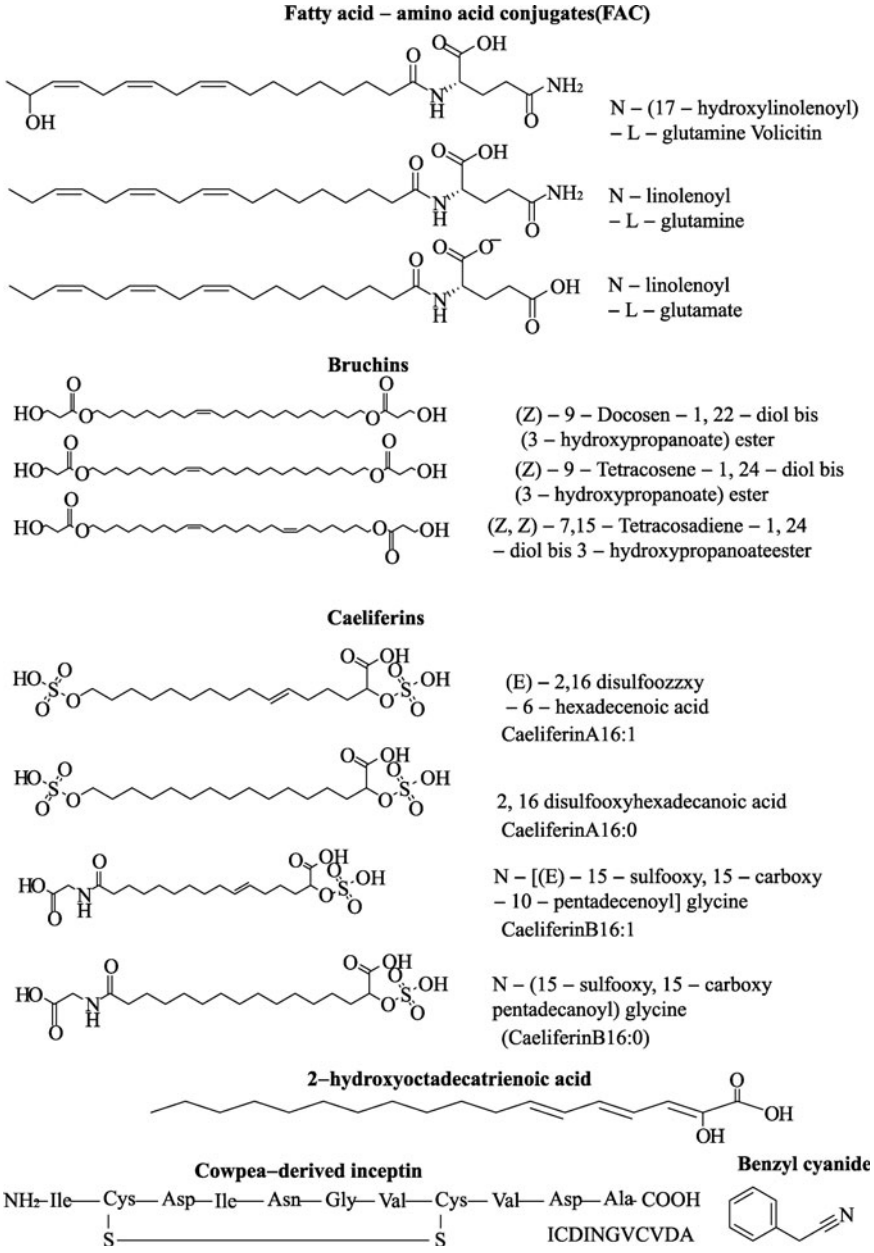

Fig. 1.1 Structures of six classes of herbivore-associated molecular patterns (HAMP).

A. Plant defense signaling molecules: plant hormones

jasmonic acid ethylene salicylic acid

B. Plant defense signaling molecules: small peptides cleaved from precursor proteins

Peptides	Sequence	Pentoses Units
Systemin	NH_2A-V-Q-S-K-P-P-S-K-R-D-P-P-K-M-Q-T-D·COOH	0
SlHypSysI	NH_2R-T-O-K-Y-T-O-O-O-O-T-S-S-S-O-T-H-Q·COOH	8–17
SlHypSysII	NH_2G-R-H-D-Y-V-A-S-O-O-O-O-K-P-Q·COOH	12–16
SlHypSysIII	NH_2G-R-H-D-S-V-L-P-O-O-S-O-K-T-D·COOH	10
NtHypSysI	NH_2R-G-A-N-L-P-O-O-S-O-A-S-S-O-O-S-K-E·COOH	9
NtHypSysII	NH_2N-R-K-P-L-S-O-O-S-O-K-P-A-D-G-Q-R-P·COOH	6
PhHypSysI	NH_2R-S-L-H-K-S-O-O-O-T-O-K-P-S-D-E-Q-G-Q·COOH	10
PhHypSysII	NH_2R-H-D-Y-H-L-S-O-O-O-A-O-K-P-A-D-H-T-G-Q·COOH	10
PhHypSysIII	NH_2R-G-K-R-L-P-O-O-A-O-E-Y-D-P-O-Y-H-Q·COOH	3–6
IbHypSysI	NH_2R-E-A-K-S-P-P-P-S-P-K-P-S-D-P-K-N-P·COOH	0
IbHypSysII	NH_2R-G-A-K-S-P-P-P-S-P-K-P-S-D-P-I-N-P·COOH	0
IbHypSysIII	NH_2R-E-P-K-S-P-P-P-S-P-K-P-S-D-P-K-N-P·COOH	0
IbHypSysIV	NH_2R-E-E-K-P-O-O-O-A-O-E-T-D-D-P-N-R-P·COOH	6–12
IbHypSysV	NH_2R-E-A-R-S-P-P-P-A-P-E-K-D-I-P-T-H-P·COOH	0
IbHypSysVI	NH_2R-T-A-R-P-P-P-P-A-P-K-P-A-A-P-I-H-P·COOH	0
AtPep1	NH_2A-T-K-V-K-A-K-Q-R-G-K-E-K-V-S-S-G-R-P-G-Q-H-N·COOH	0

Fig. 1.2 Structures of plant defense signaling phytohormones (A) and small peptide hormones (B).Systemin and HypSys are aligned with their conserved central proline (P)- or hydroxyproline (O)-rich motif boxed. Sl = tomato (*Solanum lycopersicum*), Nt = tobacco (*Nicotiana tabacum*), ph = petunia (*Petunia hybrid*), Ib = sweet potato (*Ipomoea batatas*). Tomato, tobacco, and petunia are members of the Solanaceae family, whereas sweet potato belongs to the Convolvulaceae family.

Baldwin 2002; Schmelz et al. 2003a, 2003b) (Fig. 1.2). The plant defense signaling compounds then switch on the expression of an array of defense proteins involved in the production of defense end products, including allelochemicals, protease inhibitors, indigestible proteins, and volatile organic compounds (VOC) (Paré & Tumlinson 1999; Schmelz et al. 2003a, 2003b; Felton 2005). The volatile semiochemicals may serve to repel herbivores (De Moraes et al. 2001), or to recruit natural enemies as indirect defenses (De Moraes et al. 1998; Thaler 1999; Heil 2008; Dick 2009).

In response to plant defenses, herbivores may perceive plant defense signaling molecules (Li et al. 2002a), allelochemicals (Gatehouse 2002; Li et al. 2002b, 2007), and protease inhibitors (Giri et al. 1998; Zhu-Salzman et al. 2003; Moon et al. 2004), to up-regulate their digestive enzymes (De Leo et al. 1998; Cloutier et al. 2000; Mazumdar-Leighton & Broadway 2001), detoxification enzymes including cytochrome P450 monooxygenases (P450s) (Snyder et al. 1995a; Schuler 1996; Danielson 1997; Stevens et al. 2000; Li et al. 2002a, 2002b, 2007), esterases and glutathione S-transferases (GSTs) (Yu & Hsu 1985; Snyder et al. 1995b; Yu 1996, 1999; Ni & Quisenberry 2003), or to recruit more individuals of the same species by releasing aggregation pheromones (e.g. pine beetle) for counterdefense. Thus, individuals of the two species continually adjust their defenses or counterdefenses in response to their interaction partners in a reciprocal fashion that escalates over ecological time (Agrawal 2001).

Clearly, signal detection and responses are fundamental to this on-going phenotypic arms race. All reciprocal responses are stimulated by a chemical or physical signal, from the interaction partner, the organism itself, or the environment; and all signals, internal or external, must be received and processed at the level of individual cells. The genetic machinery responsible for perceiving and responding to cues constitutes the defense (in the case of a plant) or counterdefense (in the case of an insect) signaling pathways that channel extracellular information to the genome. The extracellular information then specifically transcribes defense- or counterdefense-related genes into transcripts (transcriptome) to be translated into proteins (proteome). Collectively, the composition and content of the transcriptome and proteome determine the defense or counterdefense phenotypes in the interacting species.

1.3 Signal perceiving and transduction in plants

1.3.1 Herbivore-derived signals

When attacked by herbivorous insects, plants perceive at least two types of signals–mechanical wounding/injury (specific patterns of wounding) and chemical and enzymatic cues present in insect oral secretions (OS) or oviposition fluid (OF), the two fluids from chewing herbivores that commonly come into contact with the wounded plant tissue. Mechanical wounding appears to be a general signal common to all chewing insects, whereas chemical and enzymatic cues are herbivore-specific elicitors (Gatehouse 2002). In parallel with the term-pathogen associated molecular pattern (PAMP)-used for pathogen-derived signals or elicitors in plant-pathogen interaction, the chemical and enzymatic signals identified from herbivore OS and OF are also denoted by herbivore-associated molecular patterns (HAMPs) (Felton and Tumlinson 2008; Mithöfer and Boland 2008). Phloem sap-sucking insects such as aphids and whiteflies have a feeding habit that minimizes tissue damage, and thus reduces or avoids the wounding-elicited response in plants. Instead, they often elicit a plant defense

response typical of pathogen-induced responses (Walling 2000; Zarate et al. 2007). This makes sense since whiteflies and aphids often act as vectors for plant pathogens. For these homopterans, chemical and enzymatic cues in their OF and OS are probably the only source of signals. Whether the chemical and enzymatic signals that plants perceive from these sucking herbivores are derived from the herbivores themselves, pathogens they vector, or both, remains unknown because no signals have been identified yet from sucking insects.

In contrast, six classes of chemical signals (Fig. 1.1) and a few enzymatic signals have been characterized from a number of chewing herbivores. Among the enzymatic or protein HAMPs identified so far are β-glucosidase from the OS *of Pieris brassicae* larvae (Mattiacci et al. 1995) and glucose oxidase from the OS of *Helicoverpa zea* larvae (Musser et al. 2002, 2005). β-glucosidase triggers the release of volatiles from cabbage (*Brassica capitata*) leaves (Mattiacci et al. 1995), whereas glucose oxidase suppresses the wound-induced accumulation of nicotine in tobacco (*Nicotiana tabacum*) and of trypsin inhibitor in tomato (*Lycopersicon esculentum*) (Musser et al. 2002, 2005). Meanwhile, glucose oxidase induces the production of the SA-mediated pathogenesis-related protein 1a (PR-1a) in tobacco (Musser et al. 2005).

The most well-known class of chemical HAMP are fatty acid (FA)-amino acid conjugates (FACs), represented by volicitin (Fig. 1.1) from *Spodoptera exuiga* regurgitate (Alborn et al. 1997; Weissbecker et al. 1999; Frey et al. 2000; Shen et al. 2000). FACs are synthesized in the insect gut by conjugation of host-derived FAs to amino acids (Spiteller et al. 2000; Gaquerel et al. 2009) and found in the OS of many lepidopteran larvae, including *Manduca sexta* (Halitschke et al. 2001), *Heliothis virescens* and *Helicoverpa zea* (Mori et al. 2001), *Spodoptera littoralis* (Maffei et al. 2004) and other lepidopteran larvae (Pohenert et al. 1999; Spiteller & Boland 2003). FACs have been also isolated from three non-lepidopteran species including two closely related cricket species *Teleogryllus taiwanemma* and *Teleogryllus emma* and the fruit fly (*Drosophila melanogaster*) (Yoshinaga et al. 2007). While the amino acid component of FACs is always Gln (Glu in *M. sexta*), the FA moiety of FACs varies and can be linolenic acid (C18∶3), linoleic acid (18∶2), or derivatives thereof, depending on the food plant (Alborn et al. 1997; Paré et al. 1998; Pohnert et al. 1999; Spiteller and Boland 2003; Spiteller et al. 2004; Mithöfer and Boland 2008). FACs elicit emission of volatiles from some plants such as maize (Alborn et al. 1997) and *N. attenuata* (Halitschke et al. 2001; Gaquerel et al. 2009), but not lima bean and cowpea (*Vigna unguiculata*) (Spiteller et al. 2001). FACs are also responsible for eliciting a large portion of the hundreds of genes regulated during the plant-herbivore interaction (Halitschke et al. 2003; Roda et al. 2004) as well as the reconfiguration of the proteome (Giri et al. 2006).

The remaining five classes of chemical HAMP are inceptin, caeliferins, 2-hydroxyoctadecatrienoic acid, bruchins, and benzyl cyanide (Fig. 1.1). Inceptin is an 11 amino acid peptide resulted from the proteolytic digestion of the cowpea chloroplastic ATP synthase γ-subunit (cATPC) in the midgut of the fall

armyworm (*Spodoptera frugiperda*) (Schmelz et al. 2006, 2007). It triggers the production and release of VOC production (Schmelz et al. 2006; Carroll et al. 2008). Caeliferins are saturated and monounsaturated sulfated α-hydroxy fatty acids of 15–20 carbons with their ω-carbon functionalized with either a sulfated hydroxyl or a carboxyl conjugated to glycine via an amide bond (Fig. 1.1) (Alborn et al. 2007; Mithöfer and Boland 2008). Caeliferins were isolated from the OS of the American bird grasshopper (*Schistocerca americana*) and can trigger VOC emission in maize (Alborn et al. 2007). 2-Hydroxyoctadecatrienoic acid (2-HOT) is a newly identified HMAP from the OS of *M. sexta* (Gaquerel et al. 2009). It is derived from linolenic acid through the action of the tobacco's α-dioxygenase (α-DOX) in the *M. sexta* midgut (Gaquerel et al. 2009). It allows tobacco to monitor the progression of the caterpillar's attack and to sustain its production of JA (Gaquerel et al. 2009), the central hormone that coordinates antiherbivore defenses. Benzyl cyanide is a HAMP recently characterized from *Pieris brassicae* OF (Fatouros et al. 2008). But it is a male-derived anti-aphrodisaic pheromone that is transferred to females during mating. In addition to its anti-aphrodisaic role, benzyl cyanide also acts as an elicitor for Brussels sprouts' indirect defense, and a kairomone for the egg parasitoid *Trichogramma brassicae* by attracting it to mated *P. brassicae* females (Fatouros et al. 2005, 2008). Bruchins are long chain α,ω-diols mono- and diesterified with 3-hydroxypropanoic acid (Fig. 1.1; Doss et al. 2000; Mithöfer and Boland 2008). They were isolated from the pea weevil (*Bruchus pisorum*) and cowpea weevil (*Callosobruchus maculatus*) OF. They can initiate neoplastic growth on pods of certain pea (*Pisum sativum)* genotypes at the site of egg attachment. This growth lifts the eggs above the oviposition site and thus prevents larval entry into the pod tissue and exposes the neonates to enemies and desiccation (Doss et al. 2000; Mithöfer and Boland 2008). Bruchins can also induce the expression of *CYP93C18*, a putative isoflavone synthase gene, and the accumulation of the isoflavonoid phytoalexin pisatin (Cooper et al. 2005).

1.3.2 Endogenous plant defense signals

While herbivore wounding and HAMPs described above are the initial signals triggering the escalation of plant defense phenotype, the ultimate activation of plant defense genes and the increased production of the defensive end products (e.g. allelochemicals, toxic proteins, and VOC) are mediated proximally by endogenous plant defense signals produced within seconds to minutes after recognition of herbivore-derived signals. Among the earliest signals detectable are ion fluxes, changes in plasma transmembrane potential (Vm), followed by changes in the intracellular Ca^{2+} concentration and the generation of hydrogen peroxide (H_2O_2) and nitric oxide (NO) (Maffei et al. 2007; Wu & Baldwin 2009; Howe and Jander 2008). More proximal signals are plant defense signaling molecules (Fig. 1.2) that are produced within minutes after the onset of insect herbivory (Maffei et al. 2007).

There are two groups of plant defense signaling molecules (Fig. 1.2). One group are plant peptide hormones (Fig. 1.2B) including proline-rich systemin (Pearce et al. 1991), hydroxyproline-rich glycopeptides (HypSys peptide) (Narváez-Vásquez et al. 2007; Pearce et al. 2001, 2007; Pearce and Ryan 2003) from solanaceous plants and plants outside the Solanaceae family (Chen et al. 2008), and AtPep1 peptide from *Arabidopsis* (Huffaker et al. 2006; Huffaker and Ryan 2007); all of which are 15–23 amino acids in length and processed from their precursor proteins (Bari and Jones 2009). But HypSys pepetides are unique from Systemin, AtPepe1 and other plant peptide signals, being processed from polyprotein precursors: 2 from a tobacco precursor, 3 from a tomato precursor, and 6 from a sweet potato precursor (Chen et al. 2008). Besides, HypSys peptides are often glycopeptides containing a carbohydrate moiety (Fig. 1.2B). Systemin and HypSys pepetides are included in a functionally-defined Systemin family because they share a common proline or hydroxyproline-rich central core motif (boxed in Fig 2B) and have similar functional roles in defense signaling (Narváez-Vásquez et al. 2007). Systemin and HypSys peptide are found mainly in the Solanaceae family, whereas AtPep1 and its paraglogs in *Arabidopsis* have orthologs throughout the plant kingdom (Huffaker et al. 2006; Huffaker and Ryan 2007).

Another group of plant defense signaling molecules are the well-characterized plant defense signaling hormones JA, ET and SA (Fig. 1.2A). Wounding, HAMPs, necrotrophic pathogens, and feeding by chewing herbivores often result in rapid local and systemic accumulation of JA and ET (Fig. 1.3; Caroline et al. 2007; Glazebrook 2005; Howe and Jander 2008; Maffei et al. 2007; Schmelz et al. 2009; Wu & Baldwin 2009). SA burst, on the other hand, is often induced by biotrophic and semi-biotrophic pathogens as well as phloem-sucking herbivores (Fig. 1.3; Bari and Jones 2009; Glazebrook 2005; Smith et al. 2009; Zarate et al. 2007). But there are some exceptions (Smith et al. 2009), including SA burst elicited by chewing herbivores (e.g. *Helicoverpa zea,* Bi et al. 1997) and HAMPs (e.g. inceptin, Schmelz et al. 2009) as well as JA burst induced by biotrophic pathogens (Thaler et al. 2004). Recent studies suggest that other phytohormones such as abscisic acid (ABA), auxin, gibberellic acid (GA), cytokinin (CK), and brassinosteroids (BR) are also implicated in plant defense signaling pathways (Bari and Jones 2009; Pieterse et al. 2009).

The biosynthesis pathways of the plant defense signaling hormones JA, ET, and SA have been elucidated (Catinot et al. 2008; Ogawa et al. 2006; Wasternack 2007; Wang et al. 2002; Wu and Baldwin 2009). JA is synthesized from chloroplast membrane-derived α- linolenic acid via the octadecanoid pathway that coverts α-linolenic acid to 12-oxo-phytodienoic acid (OPDA) through the chloroplastidial enzymes lipoxygenase (LOX), allene oxide synthase (AOS), and allene oxide cyclase (AOC) in the chloroplast (the filled green circle in Fig. 1.3; Browse and Howe, 2008; Wasternack 2007; Wu and Baldwin 2009). The OPDA is further transformed to JA by OPDA reductase 3 (OPR3) and three steps of β-oxidation in the peroxisome (Fig. 1.3; Wasternack 2007; Wu and Baldwin 2009).

ET is synthesized from *S*-adenosyl-L-Met through two steps: conversion of *S*-adenosyl-L-Met to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS) and oxidation of ACC to form ET by ACC oxidase (ACO) (Fig. 1.3; Wang et al. 2002; Wu and Baldwin 2009). SA can be synthesized via both the isochorismate pathway and phenylalanine pathway (Fig. 1.3; Catinot et al. 2008; Ogawa et al. 2006). The isochorismate pathway is comprised of the rate-limiting isochorismate synthase (ICS) that converts chorismate to isochorismate, and the isochorismate pyruvate lyase (IPL) that forms SA from isochorismate. The phenylalanine pathway proceeds from phenylalanine via *trans*-cinnamic acid and benzoic acid (BA) to SA, with phenylalanine ammonia lyase (PAL) catalyzing the conversion of phenylalanine to *trans*-cinnamic acid, and benzoic acid 2-hydroxylase (BA2H) converting benzoic acid to SA (Fig. 1.3; Catinot et al. 2008; Ogawa et al. 2006). The relative importance of the two SA synthesis pathways is still in debate and thus merits further studies.

1.3.3 Plant defense signaling transduction pathway

The signal transduction pathway that connects herbivore-derived signals, wounding and HAMPs (Fig. 1.1), to rapid bursts of plant defense signaling molecules (Fig. 1.2) and the ultimate activation of defense genes/phenotype has being gradually elucidated from intensive research in the model solanaceous (tomato, tobacco) and brassicaceous (*Arabidopsis*) plants in the last decade. Based on recent excellent reviews (Howe and Jander 2008; Koornneef and Pieterse 2008; Bari and Jones 2009; Pieterse et al. 2009; Wu and Baldwin 2009) and other available models and data (Alonso and Stepanova 2004; Boter et al. 2004; Dong 2004; Du et al. 2009; Durrant and Dong 2004; Fobert and Després 2005; Guo & Ecker 2004; Lorenzo et al. 2004; Pieterse and Van-Loon 2004; Ryan and Pearce 2003; Scheer and Ryan 2002; Xiao et al. 2004; Xie et al. 1998; Yaeno and Iba 2008; Yoo et al. 2008), a comprehensive schematic model of plant defense transduction pathway is proposed here (Fig. 1.3). The whole defense signaling network is comprised of three parallel yet interconnecting phytohormone signaling pathways, namely JA (the left one in Fig. 1.3), ET (the central one in Fig. 1.3) and SA (the right one in Fig. 1.3) pathways.

The JA signaling pathway can be turned on by the common mechanical wounding caused by chewing pests and elicitors derived from chewing herbivores (HAMPs) or necotrophic pathogens (PAMPs) (Fig. 1.3; Caroline et al. 2007; Glazebrook 2005; Howe and Jander 2008; Maffei et al. 2007; Schmelz et al. 2009; Wu & Baldwin 2009). Exactly how plants perceive mechanical wounding and elicitors remains elusive. In the model solanaceous plants (tomato & tobacco), wounding inevitably disrupts cellular compartments (e.g., the vacuole). As a result, prosystemin (tomato), proHypSys (tobacco or other solanaceous plants), or PROPE (*Arabidopsis*), a precursor polypeptide constitutively present at low levels in the cytoplasm (Narvaez-Vasquez and Ryan 2004) or cell wall (Narvaez-Vasquez et al. 2005) , is exposed to proteinases,

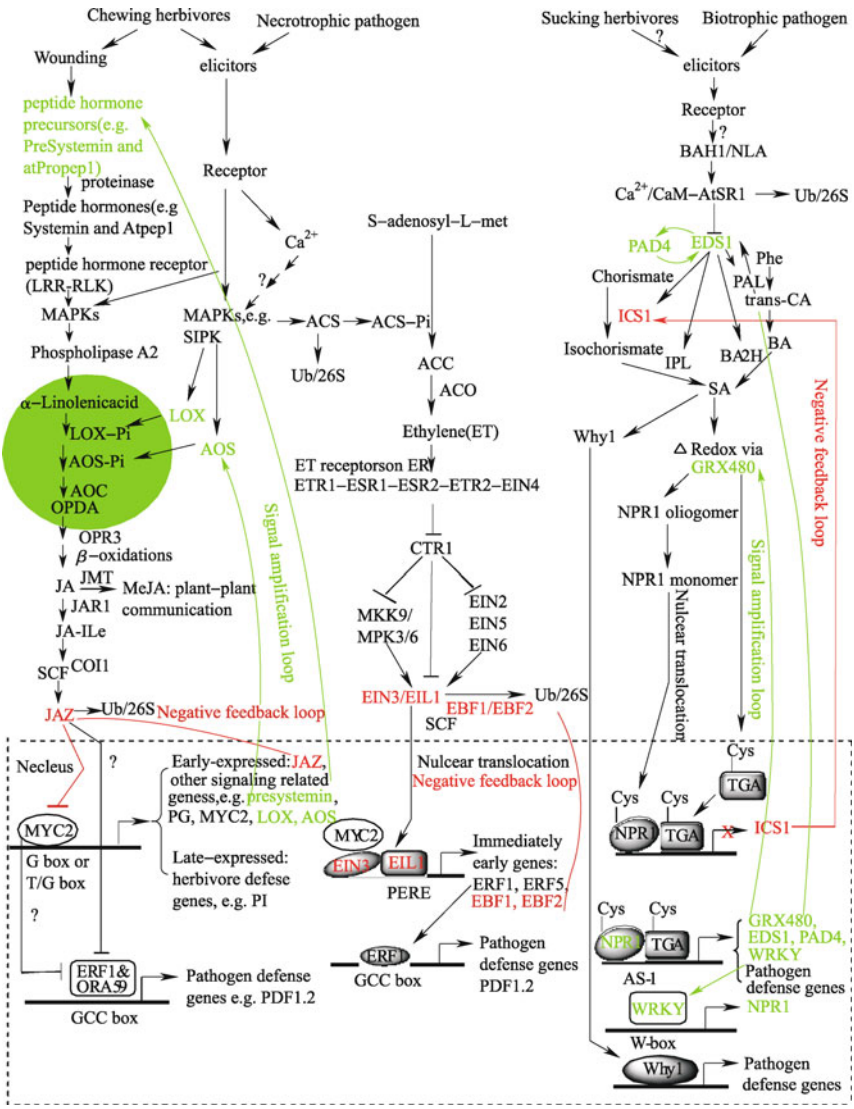
Chewing herbivores
Necrotrophic pathogen
Wounding
elicitors
peptide hormone precursors(e.g. PreSystemin and atPropep1)
proteinase
Peptide hormones(e.g Systemin and Atpep1
peptide hormone receptor (LRR-RLK)
MAPKs
Phospholipase A2
α–Linolenicacid
LOX–Pi
AOS-Pi
AOC
OPDA
OPR3
β–oxidations
JA
JMT
JAR1
JA-ILe
MeJA: plant–plant communication
SCF COI1
JAZ
Ub/26S
Negative feedback loop
Receptor
Ca2+
?
MAPKs,e.g. SIPK
LOX
AOS
Signal amplification loop
S–adenosyl–L–met
ACS
ACS–Pi
Ub/26S
ACC
ACO
Ethylene(ET)
ET receptorson ER
ETR1–ESR1–ESR2–ETR2–EIN4
CTR1
MKK9/ MPK3/6
EIN2 EIN5 EIN6
EIN3/EIL1
Ub/26S
EBF1/EBF2
SCF
Nulcear translocation
Negative feedback loop
Necleus
?
MYC2
G box or T/G box
?
Early-expressed:JAZ, other signaling related geness,e.g. presystemin, PG, MYC2, LOX, AOS
Late–expressed: herbivore defese genes, e.g. PI
ERF1&ORA59
GCC box
Pathogen defense genes e.g. PDF1.2
MYC2
EIN3
EIL1
PERE
Immediately early genes: ERF1, ERF5, EBF1, EBF2
ERF1
GCC box
Pathogen defense genes PDF1.2
Sucking herbivores
Biotrophic pathogen
?
elicitors
Receptor
?
BAH1/NLA
Ca2+/CaM–AtSR1
Ub/26S
PAD4
EDS1
Phe
PAL
trans-CA
Chorismate
ICS1
Isochorismate
IPL
BA2H
BA
SA
Why1
Δ Redox via GRX480
NPR1 oligomer
NPR1 monomer
Nulcear translocation
Signal amplification loop
Negative feedback loop
Cys
TGA
Cys
Cys
NPR1
TGA
X
ICS1
Cys
Cys
NPR1
TGA
GRX480, EDS1, PAD4, WRKY
Pathogen defense genes
AS-1
WRKY
NPR1
W-box
Why1
Pathogen defense genes

Fig. 1.3 A proposed model showing the activation of the JA, ethylene, and SA signaling pathways in response to wounding, herbivore and pathogen attack. biosynthesis and signaling pathways of JA, SA, and ET) plant defense signaling pathways.Attack by herbivore or pathogen often results in rapid synthesis and accumulation of jasmonic acid (JA), ethylene (ET), or/and salicylic acid (SA), which in turn activate the corresponding JA (the left one), ET (the central one), or SA (the right one) signaling pathways. SA signaling pathway generally activates plant defense responses against biotrophic and hemi-biotrophic pathogens. By contrast, JA and ET signaling pathways are usually associated with defense against necrotrophic pathogens and herbivorous insects. Arrows indicate activation or positive interaction, whereas blocked lines indicate repression or negative interaction. Loops that are comprised of green pathway components and green line/curves represent positive signaling amplification loops, whereas loops of red pathway components and red lines/curves represent negative feedback loops. LRR-RLK receptor, leucine-rich repeat receptor-like kinases receptor; MAPKs, mitogen-activated protein kinases; SIPK, salicylic acid-induced protein kinase; LOX, lipoxygenase; LOX-Pi, phosphorylated LOX; AOS, allene oxide synthase; AOC, allene oxide cyclase; OPDA, 12-oxo-phytodienoic acid; OPR3, OPDA reductase 3; JMT, JA carboxyl methyltransferase; MeJA, methyl JA; JAR1, JASMONATE RESISTANT 1; JA-Ile, jasmonoyl-isoleucine; SCF, Skp, Cullin, F-box;COI1, CORONATINE INSENSITIVE 1; JAZ, jasmonate ZIM-domain; Ub/26S, the ubiquitin/26S proteasome; PG, polygalacturonase; PI, protease inhibitors; PDF1.2, PLANT DEFENSIN1.2; ACC, amino-cyclopropane-1-carboxylic acid; ACS, ACC synthase; ACO, ACC oxidase; ER, endoplasmic reticulum; ETR1, Ethylene receptor 1; ETR2, Ethylene receptor 2; ESR1, Ethylene response sensor 1; ESR2, Ethylene response sensor 2; EIN4, Ethylene insensitive 4; CTR1, constitutive triple response1; EIN2, Ethylene insensitive 2; EIN5, Ethylene insensitive 5; EIN6, Ethylene insensitive 6; EIN3, Ethylene insensitive 3; EIL1, EIN3-like 1; EBF1/EBF2, EIN3-binding F-box protein 1 and 2; PERE, primary ethylene response element; ERF1, ETHYLENE RESPONSE FACTOR 1; BAH1/NLA, benzoic acid hypersensitive1 / nitrogen limitation adaptation; CaM, Calmodulin; AtSR1, *Arabidopsis thaliana* signal-responsive gene 1; EDS1, enhanced disease susceptibility 1; PAD4, phytoalexin–deficient 4; ICS1, isochorismate synthase 1; IPL, isochorismate pyruvate lyase; Phe, phenylalanine; *trans*-CA, *trans*-cinnamic acid; BA, benzoic acid; PAL, phenylalanine ammonia lyase; BA2H, benzoic acid 2-hydroxylase; NPR1, NONEXPRESSOR OF PATHOGENESIS-RELATED (PR) GENES 1; Why1, whirly 1; AS-1 element, activation sequence 1 element.

either from the disrupted plant cells or from the insect OS or OF. This leads to proteolytic release of the active plant defense peptide hormones (e.g. Systemin in tomato, HypSys in tobacco, or AtPep1 in *Arabidopsis*) (Ryan & Pearce 1998; Ryan 2000; Ryan & Pearce 2003; Huffaker et al. 2006). Peptide hormones (e.g. Systemin) then bind to their membrane-bound LRR-RLK (leucine-rich repeat receptor- like kinases) receptors (Scheer & Ryan 2002; Yamaguchi et al. 2006), leading to phospholipase A2-mediated release of α-linolenic acid from chloroplast membrane lipids via a mitogen-activated protein kinase (MAPK) cascade. α-Linolenic acid is converted into JA via the octadecanoid pathway composed of LOX, AOS, and AOC, followed by OPR3 reduction and three β-oxidation reactions in peroxisome (Fig. 1.3). Herbivore- or necrotrophic pathogen- derived elicitors further amplify the wounding-elicited JA burst by activating the phospholipase A2-mediated release of α-linolenic acid (JA precursor) from chloroplast membrane or phosphorylating the JA biosynthesis enzymes LOX and AOS through their receptor-activated MAPK cascade (Fig. 1.3). The produced JA is converted to methyl jasmonate (MeJA) by jasmonic acid carboxyl methyltransferase (JMT) for plant-plant communication (Farmer and Ryan, 1990) and/or to the bioactive JA molecule JA-isoleucine (JA-Ile) by JAR1 (jasmonate resistant 1), which encodes a JA amino acid synthetase (Staswick and Tiryaki 2004; Thines et al. 2007). JA-Ile specifically binds to its putative receptor, the F-box protein coronatine insensitive 1(COI1; Xie et al. 1998; Katsir et al. 2008), leading to the activation of the E3 ubiquitin ligase SCF^{COI1} (SKP1/cullin/F-box protein; COI1 is the F-box protein), which targets jasmonate ZIM-domain (JAZ) proteins for ubiquitination and subsequent degradation by the 26S proteasome (1.3; Chini et al. 2007; Thines et al. 2007). JAZ proteins are negative regulators that constitutively repress a set of transcription factors such as the basic helix-loop-helix (bHLH) MYC2, and the ethylene-response-factor1 (ERF1) and ORA59 and inhibit the expression of JA-responsive genes (Lorenzo et al. 2003, 2004; Boter et al. 2004; Chini et al. 2007; Thines et al. 2007). The degradation of JAZ proteins allows the above transcription factors to antagonistically regulate the expression of two groups of JA-induced genes, i.e., the T/G box-containing early-expressed JA signaling (e. g., JAZ, LOX, MYC2, etc.) and late-expressed herbivore defense/ wound-responsive genes by MYC2, and the GCC box-containing necrotrophic pathogen defense genes (resistance to necrotrophic pathogen) by ERF1 and ORA59, leading to elevated defenses against herbivores and pathogens (Boter et al. 2004; Lorenzo et al. 2004; Lorenzo and Solano 2005; Dombrecht et al. 2007; Chini et al. 2007; Thines et al. 2007; Bari and Jones 2009).

Elicitors from chewing herbivores and necrotrophic pathogens also activate the ET signaling pathway by phosphorylating and stabilizing the ET biosynthetic enzymes aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) via their receptor-activated MAPK cascade (Fig. 1.3; Liu & Zhang 2004). If not phosphorylated, ACS will be degraded by the 26S proteasome. The accumulated ET then binds to the sensor domains of a family of 5 endoplasmic reticulum

(ER)–localized ET receptors [ETR1, ETR2, EIN4 (ethylene insensitive4), ERS1 (ethylene response sensor1), and ERS2], which are constitutively active with their histine kinase domain interacting with the N-terminal domain of the negative regulator constitutive triple response1 (CTR1), a Raf-like serine-threonine kinase (Guo & Ecker 2004; Alonso & Stepanova 2004). Association of CTR1 with the ER-localized ET Receptors is required for the repression of the downstream positive regulators EIN2/EIN5/EIN6, MAPK cascade (MKK9-MPK3/MPK6), and the transcription factors EIN3/EIL1 (EIN3-like 1) (Fig. 1.3; Guo & Ecker 2004; Alonso & Stepanova 2004; Yoo et al. 2008). CTR1 can phosphorylate the positive transcription factor EIN3 at Thr 592, promoting the degradation of EIN3 by the E3 ubiquitin ligase $SCF^{EBF1/EBF2}$ (SKP1/cullin/F-box protein; EBF1 and EBF2 are the F-box proteins) and the 26S proteasome (Yoo et al. 2008). It can also repress EIN3/EIL by inhibiting the positive regulators EIN2/EIN5/EIN6 (Guo & Ecker 2004; Alonso & Stepanova 2004**).** Furthermore, it can repress ET signaling by inactivating the MAPK cascade comprising MKK9 and MKP3/MPK6 whose function is to stabilize EIN3 and promote its nuclear translocation by phosphorylating EIN3 at Thr 174 (Yoo et al. 2008). Binding of ET to ER-associated ET receptors inactivates ET receptors, presumably by inducing a conformational change. This in turn leads to the dissociation of the immediate downstream negative regulator CTR1 from the ET receptors and thus the inactivation of CTR1. As a result, the CTR1-mediated repression of the downstream positive regulators including the EIN2- EIN5-EIN6 cascade, the MKK9-MPK3/MPK6 cascade and the transcription factor EIN3 is relieved, leading to the stabilization and nuclear translocation/accumulation of the transcription factors EIN3/EIL1. EIN3/EIL1, probably together with JA-induced transcription factors such as MYC2, promote the expression of the immediate early ET-response genes such as the transcription factor ERF1 by binding to the primary ethylene response element (PERE) in their promoter regions. ERF1 in turn induces the expression of the GCC box-containing necrotrophic pathogen defense genes (Guo & Ecker 2004; Alonso & Stepanova 2004; Yoo et al. 2008).

The SA signaling pathway is believed to be activated by the elicitors from biotrophic pathogens and phloem sap-sucking herbivores (Fig. 1.3). Based on two recent studies (Du et al. 2009; Yaeno and Iba 2008), we propose that binding of the herbivore- or biotrophic pathogen-derived elicitors to their receptors activates BAH1/ NLA (benzoic acid hypersensitive1 / nitrogen limitation adaptation), a RING-type ubiquitin E3 ligase (Yaeno and Iba 2008). This in turn leads to the 26S proteasome-mediated degradation of the transcription repressor complex Ca^{2+}/Calmodulin(CaM) /AtSR1 that constitutively represses the expression of the downstream transcription factor enhanced disease susceptibility1 (EDS1) by competing for the CGCG box with unidentified transcription activators in the EDS1promoter (Du et al. 2009). Consequently, EDS1 is expressed, which in turn triggers the EDS1-PAD4 (phytoalexin-deficient4; another positive transcription factor) positive feedback loop whereby EDS1 and PAD4 reciprocally drive the expression of each other (Fig. 1.3; Du et al. 2009).

EDS1 and PAD4 then promote the expression of the SA biosynthetic enzymes in both the isochorismate (ICS1, IPL) and phenylalanine (PAL, BA2H) pathways, resulting in an SA burst (Fig. 1.3; Du et al. 2009; Durrant and Dong, 2004). SA burst induces the glutaredoxin GRX480-meidated oxidoreduction (redox) change, which reduces the conserved key cysteine residues in the TGA transcription factors and their redox-sensitive co-activator nonexpressor of pathogenesis-related (PR) genes 1 (NPR1) (Mou et al. 2003; Durrant and Dong 2004; Dong 2004; Fobert and Després 2005; Ndamukong et al. 2007; Tada et al. 2008). NPR1 is constitutively present in the cytoplasm as an oligomer formed through intermolecular disulfide bonds. GRX480-mediated reduction of Cys 82 and Cys 216 in NPR1 leads to its monomerization and nucleocytoplasmic localization, which is required for the activation of pathogenesis-related (PR) genes (NPR1 monomer in nucleus) and SA repression of the JA signaling (NPR1 monomer in cytoplasm). Reduction of conserved cysteines in TGA transcription factors enables their interaction with monomeric NPR1, leading to repression of ICS1 and expression of the activation sequence 1 element (AS-1 element)-containing genes, including PR genes and SA signaling genes (Fig. 1.3; Wildermuth et al. 2001; Ogawa et al. 2007; Mou et al. 2003; Durrant and Dong 2004; Dong 2004; Fobert and Després 2005). In addition, SA burst can also directly activate the whirly (why) family transcription factors such as why1 and thus induce expression of some PR genes in a NPR1-independent manner (Durrant and Dong 2004).

1.3.4 The features of the plant defense signaling pathway

While the plant defense signaling pathway is described as three (JA, ET and SA) parallel linear pathways (Fig. 1.3), the three pathways actually cross-talk at multiple nodes, forming a signaling network that fine-tunes plant growth and defense in response to plant attackers (Bari and Jone 2009; Koornneef and Pieterse 2008). JA and ET signaling pathways can be triggered by the same signals such as HAMP and elicitors from necrotrophic pathogens and may act synergistically (when both pathways are activated) or antagonistically (when only JA pathway is activated) to modulate plant defense against necrotrophic pathogens and herbivorous insects. The JA-ET interactions are largely mediated by the positive transcription factors ERF1 and MYC2. ERF1 integrates signals from JA and ET via the JA-activated MYC2 (necessary for ERF1's full expression) and the ET-activated EIN3/EIL1 (always required for ERF1's expression) respectively (see Fig. 1.3) and functions as a positive regulator of JA and ET signaling for pathogen defense genes (Lorenzo et al. 2003; Bari and Jone 2009; Koornneef and Pieterse 2008). MYC2, on the other hand, induces JA mediated expression of the G box or T/G box-containing wound/herbivore response genes but represses the expression of the GCC box-containing necrotrophic pathogen defense genes (Fig. 1.3; Lorenzo and Solano 2005;

Dombrecht et al. 2007). When both JA and ET pathway are activated, MYC2 also induces the full expression of ERF1, which in turn promotes expression of the JA-mediated pathogen defense genes.

SA pathway is usually associated with plant defense against biotrophic and hemi-biotrophic pathogens and cross talks antagonistically with JA pathway. The key signaling node of the JA-SA antagonism is the redox-sensitive co-activator NPR1, whose redox-mediated conformational transition (oligomer vs. monomer) and nucleocytoplasmic translocation determine which pathway is to be activated. Oligomeric NPR1 negatively regulates SA production during herbivore attack and thus suppress SA/JA cross talk to allow induction of JA-mediated defenses against herbivores (Koornneef and Pieterse 2008). Monomeric NPR1, on the other hand, is required for SA repression of JA signaling (NPR1 monomer in cytoplasm) and the activation of SA-mediated defense against pathogens (NPR1 monomer in nucleus) (Dong 2004; Pieterse and Van Loon 2004; Spoel et al. 2003; 2007; Koornneef and Pieterse 2008; Yuan et al. 2007; Koornneef et al. 2008). But ET burst can render SA repression of JA signaling NPR1 independent (Leon-Reyes et al. 2009). Other signaling nodes modulating the JA-SA antagonism include MYC2, which acts as a negative regulator of SA signaling (Laurie-Berry et al. 2006), and GRX480 and WRKY transcription factors WRKY 62 and 70, all of which are involved in the SA-mediated suppression of JA signaling (Li et al. 2004, 2006; Mao et al. 2007; Ndamukong et al. 2007).

Besides, the plant defense signaling network also has the following four features: signaling redundancy, signal amplification loops (positive feedback loop; denoted by green text linked with green arrowed lines/curves in Fig. 3), negative feedback loops (denoted by red text linked with red arrowed lines/curves in Fig. 1.3), and destruction of negative (JAZ in JA signaling and Ca^{2+}/CaM/AtSR1 in SA signaling) or positive (EIN3/EIL1 in ET signaling) regulators via the 26S proteasome (Fig. 1.3; Ballaré 2009). Signaling redundancy can occur both at the levels of signal perception (different environmental signals trigger similar plant responses) and signaling circuits (the same signal activates parallel response channels) (Ballaré 2009). An example for the former would be the activation of the JA signaling pathway by wounding, elicitors from different chewing herbivores and necrotophic pathogens. An example for the latter would be the activation of the JA, ET, and SA signaling pathway by the same signal inceptin (Schmelz et al. 2009). Targeted destruction of negative or positive regulators via the 26S proteasome allows plants to rapidly escalate their defense against attackers by de-repressing temporally inactivated but otherwise fully functional signaling circuits (Ballaré 2009). Co-existence of both positive and negative feedback loops enables plants to mount a defense response that is commensurate with the intensity and duration of the attack (Howe and Jander 2008). This provides host plants with a mechanism to allocate resources between growth/reproduction and defense against herbivores and pathogens.

1.4 Signal perception and transduction in herbivorous insects

When feeding on plants, herbivorous insects not only provide cues for plants to gear up plant defenses, but also obtain signals from plants to activate their own counterdefense. A great deal of evidence indicates that plant defense signaling hormones (Li et al. 2002a) and plant defense compounds such as toxic allelochemicals and protease inhibitors are signals that insects detect and use to upregulate their counterdefense genes (Li et al. 2002b; Moon et al. 2004; Zhu-Salzman et al. 2003). Toxic allelochemicals often induce a number of detoxification enzymes including cytochrome P450 monooxygenases (P450s) (Yu 1982; Schuler 1996; Snyder et al. 1995; Stevens et al. 2000; Danielson 1997; Li et al. 2002b, 2007), glutathione-S-transferases (GSTs) (Yu 1996, 1999; Snyder et al. 1995; Li 2009) and esterases (Yu & Hsu 1985), which metabolize and detoxify these toxic allelochemicals. Protease inhibitors,on the other hand, elicit the overproduction of existing digestive enzymes (De Leo et al. 1998) and expression of protease inhibitor-insensitive digestive enzymes (Cloutier et al. 2000; Mazumdar-Leighton & Broadway, 2001), hydrolyzing enzymes that fragment the inhibitors (Giri et al. 1998; Zhu-Salzman et al. 2003), and even P450s (Moon et al. 2004). Little, however, is known about how insects perceive and transduce these signals into elevated counterdefense phenotypes.

Perhaps, the only allelochemical transduction cascade that has been extensively studied in insects is the xanthotoxin response cascade mediating the upregulation of *CYP6B1* in *Papilio polyxenes,* a specialist, and *CYP6B4* in *P. glaucus*, a generalist (Brown et al. 2005; McDonnell et al. 2004; Petersen et al. 2003). Despite divergence in their coding sequences and furanocoumarin-metabolizing capabilities, the promoter sequences of the *CYP6B4* and *CYP6B1* genes are highly conserved in a number of sequences identified as response elements in other invertebrates and vertebrates. For example, both of the *CYP6B1* and *CYP6B4* promoter sequences contain an overlapping EcRE/ARE/XRE-xan element (Petersen et al. 2003; Brown et al. 2005; McDonnell et al. 2004). They also contain putative XRE-AhR elements similar to those found in mammalian P450 promoters that are activated by binding to activated aryl hydrocarbon receptor (AhR)–ARNT complexes. Both the overlapping EcRE/ARE/XRE-xan element and the XRE-AhR element are necessary for basal and xanthotoxin-inducible expression of the *CYP6B1* (Petersen et al. 2003; Brown et al. 2005). In comparison, the EcRE/ARE/XRE-xan element is necessary for *CYP6B4* induction by xanthotoxin but not for its minimal basal expression (McDonnell 2004). Recently, Brown et al. (2005) showed that Spineless (Ss) and tango (Tgo) proteins, the *Drosophila melanogaster* homologues of mammalian AhR and ARNT, enhanced basal expression of the *CYP6B1* promoter but not the magnitude of its xanthotoxin and benzo[a]pyrene induction. Other components of the xanthotoxin transduction cascade, including transcription factors remains unknown.

The only protease inhibitor transduction cascade that has been studied in

insects is the soybean cysteine protease inhibitor soyacystatin N (scN) response cascade mediating the activation of the scN-insensitive cathepsin B-like cysteine protease called *CmCatB* in the cowpea bruchid (*Callosobruchus maculatus*) (Ahn et al. 2007). In the absence of the scN signal, *CmCatB* expression is negatively regulated by the *C.maculatus* nuclear receptor Seven-up (CmSvp) through its binding to the two tandem chicken ovalbumin upstream promoter (COUP) elements in the *CmCatB* promoter. In response to scN-containing diets, the protein level of CmSvp is significantly reduced, leading to the de-repression of the expression of CmCatB (Ahn et al. 2007). More experiments are needed to reveal the scN transduction pathway, including how the protein level of CmSvp is reduced and what directly triggers the process.

1.5 Herbivore-induced plant defense transcriptomes and proteomes

Phenotypic changes in defenses and counterdefenses of individual plants and insects are mediated by regulation of gene expression in both organisms. Although some genes (e.g. LOX, VSP, PDF) have been known to be associated with herbivore attack, wounding, JA or SA treatment for an extended period, characterization of a broader transcriptional change in response to herbivore attack in plants has only recently been made feasible by the development of genomic transcript profiling methods. The first microarray study of plant-insect interactions compared the expression of 150 pre-selected defense-related genes in *Arabidopsis* plants mechanically wounded or challenged with caterpillars of the crucifer specialist *Pieris rapae*. This revealed a difference between insect-attacked or wounded plants, particularly in the expression of dehydration-inducible genes (Reymond et al. 2000). The use of a similar microarray showed that the feeding of the green peach aphid (*Myzus persicae*) on *Arabidopsis* leaves (Col-0 ecotype) up-regulated or down-regulated genes involved in oxidative stress (GSTs, superoxide dismutases), calcium-dependent signaling, and pathogenesis-related responses (BGL2, PR-1, hevein-like protein), ethylene biosynthesis genes (ACC oxidase 1), aromatic biosynthesis genes (PAL2, chalcone synthase, tyrosine decarboxylase), and tryptophan biosynthetic pathway genes (anthranilate synthase, tryptophan synthase) (Moran et al. 2002). A study using a cDNA microarray consisting of 2,375 *Arabidopsis thaliana* genes revealed that JA treatment altered the expression of 371 genes (Schenk et al. 2000). Using a large-scale microarray covering 25%–30% of the *Arabidopsis* genome (7,200 unique genes), Reymond et al. (2004) compared the *Arabidopsis* defense transcriptomes in response to a specialist caterpillar, *Pieris rapae*, and a generalist caterpillar, *Spodoptera littoralis*. Although there are reported differences between the two species in salivary components, nearly identical transcript profiles were observed. One hundred fourteen genes potentially involved in defense were either induced (111 genes for *P. rapae*, 112 genes for *S. littoralis*) or repressed (3 genes for *P. rapae*, 2 genes for *S. littoralis*) in response