Signaling and Communication in Plants

Helen R. Irving
Christoph Gehring *Editors*

Plant Signaling Peptides

Springer

Signaling and Communication in Plants

Series Editors

František Baluška
Department of Plant Cell Biology, IZMB, University of Bonn, Kirschallee 1,
D-53115 Bonn, Germany

Jorge M. Vivanco
Center for Rhizosphere Biology, Colorado State University, 217 Shepardson Building,
Fort Collins, CO 80523-1173, USA

For further volumes:
http://www.springer.com/series/8094

Helen R. Irving • Christoph Gehring
Editors

Plant Signaling Peptides

Springer

Editors
Helen R. Irving
Monash Institute of Pharmaceutical Sciences
Monash University
Parkville
Australia

Christoph Gehring
Division of Chemistry
Life Science and Engineering
4700 King Abdullah University of Science and Technology
Thuwal
Kingdom of Saudi Arabia

ISSN 1867-9048 ISSN 1867-9056 (electronic)
ISBN 978-3-642-27602-6 ISBN 978-3-642-27603-3 (eBook)
DOI 10.1007/978-3-642-27603-3
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2012940197

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Proteins and peptides form integral parts of all living cells where their function is essential for the survival of the cell and organism. Proteins are dynamic biomolecules that function in maintaining structure, metabolism, and also cellular communication. Peptides are loosely defined as small proteins containing 50 amino acids or less. In plants, as in animals and other organisms, peptides have diverse roles and participate in communication between cells. The focus of this volume is on the diverse roles that peptides and small proteins have in intercellular and intracellular communication in plants. In part because of their immobile nature, plants have evolved a complex array of signaling molecules to facilitate their growth and development and their interactions with the environment. A vast number of different peptide molecules make an important, but until relatively recently overlooked, component among these signaling molecules. As discussed in chapter "Plant Peptide Signaling: An Evolutionary Adaptation," plant signaling peptides have evolved in several independent events with distinct and separate phylogenies to create a diverse repertoire of signaling molecules.

This volume focuses on the roles of various peptide signaling molecules in plant growth, development, defense, and homeostasis. The roles of plant peptides in growth and development are discussed in chapters "Peptides Regulating Apical Meristem Development," "Peptides Regulating Root Growth," "Peptides Regulating Plant Vascular Development," and "The S-LOCUS CYSTEINE RICH PROTEIN (SCR): A Small Peptide with a High Impact on the Evolution of Flowering Plants." Chapter "Peptides Regulating Apical Meristem Development" reviews the well-understood role of peptide signaling in the shoot apical meristem of the model plant *Arabidopsis* that in turn has led to the discovery of related peptides in other plants. In fact, the major peptide protagonist in *Arabidopsis* CLAVATA 3 (CLV3) and its maize homolog EMBRYO SURROUNDING REGION (ESR) contributed to the naming of one of the largest signaling peptide groups, the CLE peptides. CLE peptides are involved in regulating organogenesis and have roles in the root growth and development which is discussed in chapter "Peptides Regulating Root

Growth." Members of the CLE peptides are also involved in regulating the development of the vascular cambium which is reviewed in chapter "Peptides Regulating Plant Vascular Development." In addition, members of the CLE family are co-opted by legumes during the symbiosis between legumes and rhizobia bacteria, as described in chapter "The Role of Plant Peptides in Symbiotic Interactions." However, the story of peptide signaling is not restricted to one family of peptides and certainly in development is integrated with signals from other plant growth regulators. Other peptides that contribute to organogenesis and the maintenance of stem cells include phytosulfokines (PSKs), ENOD40, rapid alkalinization factors (RALFs), and the recently discovered root growth factor (RGF). Specific and novel peptides are involved in various developmental processes. The family of S-LOCUS CYSTEINE RICH PROTEINs (SCRs) has an important role as a determinant of self-incompatibility in members of the *Brassicaceae* which is discussed in chapter "The S-LOCUS CYSTEINE RICH PROTEIN (SCR): A Small Peptide with a High Impact on the Evolution of Flowering Plants." While another recently characterized small family of peptides called EPIDERMAL PATTERNING FACTORs (EPFs) are also cysteine-rich peptides that have a role in regulating stomatal development, as reviewed in chapter "Peptides Modulating Development of Specialized Cells."

Signaling peptides also function in a wide range of plant defense responses. In fact, the first signaling peptide to be discovered and characterized was systemin which induces synthesis of proteinase inhibitors in leaves as a wound response. Since then, a myriad of plant defense proteins with diverse structures have been identified. Many of these are antimicrobial proteins and include defensins, thionins, and knottin-like peptides, as described in chapter "Plant Antimicrobial Peptides." Other signaling peptides function as endogenous amplification signals of plant innate immune responses as part of the pattern and/or microbe-associated molecular pattern (PAMP/MAMP) response which is discussed in chapter "Peptides as Danger Signals: MAMPs and DAMPs." The signal exchange initiated by rhizobia employs and/or co-opts several plant signaling peptides in the host legume, as reviewed in chapter "The Role of Plant Peptides in Symbiotic Interactions." Plant signaling peptides also have roles in maintaining overall plant homeostasis in addition to organogenesis and development, and in chapter "Peptides and the Regulation of Plant Homeostasis," the role of the small protein plant natriuretic peptide is described.

As it is highly likely that to date, only a few of the signaling peptides are known, and further plant peptide signaling molecules remain to be discovered, the last section of this volume takes a practical look at methods to identify new peptides and characterize their function. Signaling peptides usually contain an N-terminal signal motif and are secreted into the extracellular matrix (apoplast) where, in some cases, they are proteolytically cleaved. Peptides such as PSK and RGF are also sulfated on tyrosine residues, and some CLE peptides are hydroxylated on proline residues before secretion. The processing of peptides is described in chapter "Processing of Peptides" along with strategies for investigating these processes. In chapter "Methods to Isolate and Identify New Plant Signaling Peptides," the principles and methods for peptide purification are discussed. For signaling peptides to be

successful as communicators in the plant, specific partners are required, and genetic and biochemical approaches to identify these partners are described in chapter "Methods to Identify New Partners of Plant Signaling Peptides." Finally, a computational approach is outlined in chapter "Computational-Based Analysis to Associate the Function of Plant Signaling Peptides with Distinct Biological Processes" where proteins co-expressed with PROPEP2 are identified.

May 2012
Parkville, Australia — Helen R. Irving
Thuwal, Kingdom of Saudi Arabia — Chris Gehring

Contents

Plant Peptide Signaling: An Evolutionary Adaptation 1
Janet I. Wheeler and Helen R. Irving

Peptides Regulating Apical Meristem Development 25
Marc Somssich and Rüdiger Simon

Peptides Regulating Root Growth 41
Margret Sauter

Peptides Regulating Plant Vascular Development 59
Hiroo Fukuda

The S-LOCUS CYSTEINE-RICH PROTEIN (SCR): A Small Peptide with A High Impact on the Evolution of Flowering Plants 77
Isabelle Fobis-Loisy, Rumen Ivanov, and Thierry Gaude

Peptides Modulating Development of Specialized Cells 93
Lee Hunt and Julie E. Gray

Plant Antimicrobial Peptides 107
Tatyana Odintsova and Tsezi Egorov

The Role of Plant Peptides in Symbiotic Interactions 135
Virginie Mortier, Ulrike Mathesius, and Sofie Goormachtig

Peptides as Danger Signals: MAMPs and DAMPs 163
Thomas Boller and Pascale Flury

Peptides and the Regulation of Plant Homeostasis 183
Chris Gehring and Helen R. Irving

Processing of Peptides 199
Renu Srivastava and Stephen H. Howell

Methods to Isolate and Identify New Plant Signaling Peptides 217
Sunil Sagar, Chris Gehring, and Kenneth P. Minneman

Methods to Identify New Partners of Plant Signaling Peptides 241
Melinka A. Butenko, Markus Albert, and Reidunn B. Aalen

Computational-Based Analysis to Associate the Function of Plant Signaling Peptides with Distinct Biological Processes 257
Stuart Meier and Lara Donaldson

Index 279

Plant Peptide Signaling: An Evolutionary Adaptation

Janet I. Wheeler and Helen R. Irving

Abstract Peptide signaling molecules are well characterized in animal systems, but it is only over the last three decades that they have been recognized in plants. In this chapter, we compare some of the major features of animal peptide signaling molecules with the new classes that have been identified in plants. We introduce the concept of modular signaling and discuss how this adaptable feature can be evolutionarily advantageous to multicellular organisms. Most signaling peptides have been identified in angiosperms (both monocot and dicot) although representative signaling peptides occur in moss and green algae. Some classes contain peptides with highly diverse sequences (within and across species) while other peptide signaling classes are small or represented by a single peptide or only found in a single family of plants. The different classes of plant signaling peptides are not phylogenetically related indicating that they have been independently selected to enable modular or "mix and match" signaling.

1 Introduction

Plants, due to their sessile nature, need to respond rapidly to fluctuations in their environment which can range from changes in humidity and temperature to predatory attacks by herbivores or pathogens. Plants successfully withstand these challenges as they have evolved complex and highly interconnected signaling networks that operate both intra- and extracellularly to relay cellular responses. The signaling processes are mediated by ligands that include gases, small organic molecules, and peptides and small proteins. Specific receptors for these molecules recognize the signal and activate signaling cascades that relay the message within

J.I. Wheeler • H.R. Irving (✉)
Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, VIC 3052, Australia
e-mail: helen.irving@monash.edu

H.R. Irving and C. Gehring (eds.), *Plant Signaling Peptides*,
Signaling and Communication in Plants 16, DOI 10.1007/978-3-642-27603-3_1,

specific cells to stimulate the appropriate physiological responses. Peptide and small protein molecules contribute as signaling ligands in a wide variety of plant functions ranging from plant cell differentiation to host defense responses. In addition, plants produce an arsenal of plant defense peptides and proteins with antimicrobial activity and many proteins that act as protease inhibitors. In this book, the contributions of various peptides and small proteins to plant growth and development are investigated. The roles of signaling peptides in modulating plant growth and development are examined in chapters "Peptides Regulating Apical Meristem Development, Peptides Regulating Root Growth, Peptides Regulating Plant Vascular Development, The S-LOCUS CYSTEINE RICH PROTEIN (SCR): A Small Peptide with a High Impact on the Evolution of Flowering Plants, and Peptides Modulating Development of Specialized Cells," and the signaling peptides that participate in defense and homeostasis are examined in chapters "Plant Antimicrobial Peptides, The Role of Plant Peptides in Symbiotic Interactions, Peptides as Danger Signals: MAMPs and DAMPs, and Peptides and the Regulation of Plant Homeostasis." Various strategies for working with plant signaling peptides are explored in chapters "Processing of Peptides, Methods to Isolate and Identify New Plant Signaling Peptides, Methods to Identify New Partners of Plant Signaling Peptides, and Computational-Based Analysis to Associate the Function of Plant Signaling Peptides with Distinct Biological Processes."

In this chapter, we introduce the secreted peptide signaling molecules and briefly review the role of the various classes of the molecules in plants and their phylogenetic relationships. In the process, we discuss several generalized themes that have emerged following recent developments in biochemical, genetic, and molecular biology studies focusing on the evolutionary adaptations that the peptides offer as a preface to the detailed discussions in the chapters following.

2 Animal Peptide Signaling Systems

The isolation of insulin revealed that proteins could act as hormones in mammals (Banting et al. 1922a, b), and since then, a plethora of signaling peptides have been discovered that act as hormones or as paracrine and/or autocrine molecules. Hormones are secreted from an exocrine gland and distributed via the bloodstream to distant regions of the body, whereas paracrine molecules are secreted and act on nearby cells in the tissue and autocrine signals act on the secreting cell. Paracrine signaling is a very ancient form of cell communication also evident in unicellular organisms as well as multicellular organisms. Unicellular organisms evolved mechanisms to respond to the presence of environmental constraints and other cells. Quorum sensing is used by bacteria to detect the presence of other bacteria and, when a certain level of bacterial population is reached (the quorum), signals the generation of various compounds including antibiotics directed at other bacteria. Gram-positive bacteria use peptide ligands to undertake quorum sensing (Antunes et al. 2010; Hibbing et al. 2010; Thoendel and Horswill 2010). Specific

peptide ligands are also secreted by haploid cells of *Saccharomyces cerevisiae* as pheromones that are recognized by the opposite haploid cell mating type (Bardwell 2004). In both cases, peptide ligands form an important part of the signaling network. This trait has been preserved through evolution as peptides form major groups of paracrine signaling molecules in animals. An example is the cytokine family of secreted small protein signals that regulate immune and hematopoietic cell development and includes interleukins, interferons, and erythropoietin. The cytokines and their receptors all have similar tertiary structural homology indicating that all the ligands are derived from a common ancestral protein as are all the receptors (Ozaki and Leonard 2002). So it is not surprising that the cytokine receptors all activate similar signaling pathways involving kinase enzymes [Janus kinase (JAK)] that in turn phosphorylate and activate transcription factors [signal transduction and activation of transcription (STAT) proteins] forming the JAK/STAT pathway (Yamaoka et al. 2004). The cellular response to a particular cytokine is dictated by whether the receptor is present and the particular group(s) of transcription factors present in that cell. Thus, a type of mix and match of ligand and receptor class modules has coevolved with transcription factors to modulate gene transcription to regulate cell development.

3 Discovery of Peptide Signaling Systems in Plants

Over the last two decades, the number of known peptide signaling molecules has increased dramatically from the first molecule identified in tomato to now over 15 diverse families that influence plant growth and development. A list of representatives of peptide signaling families found in *Arabidopsis thaliana* and a brief description of their known function(s) are presented in Table 1. Systemin is an 18-amino-acid peptide which was the first peptide signaling molecule identified as the factor that induced synthesis of proteinase inhibitors in wounded tomato leaves (*Solanum lycopersicum*) (Pearce et al. 1991). Systemin obtained its name as it was associated with a systemic response where proteinase inhibitors were also produced in tomato leaves above the wounded leaves. Prosystemin is the precursor peptide containing the systemin peptide within its sequence (McGurl et al. 1992). Overexpression of prosystemin in tomato induces the systemic response involving protease inhibitors (McGurl et al. 1994). The systemic response involving systemin is entwined with the production of the lipid-derived hormone jasmonic acid which is also associated with the wound response (Farmer et al. 1992; Farmer and Ryan 1992). Grafting experiments revealed that synthesis of jasmonic acid and systemin in the lower leaves at the site of wounding is necessary for strong upregulation of proteinase inhibitors in the upper leaves following perception of jasmonic acid (for a review, see Schilmiller and Howe 2005). Systemin is restricted to members of the Solanaceae and hence is not listed in Table 1.

Small peptides secreted into plant cell culture media were also identified about this period. The first identified was phytosulfokine (PSK) which is a pentapeptide

Table 1 Summary of plant peptide signaling molecules present in *Arabidopsis thaliana*

Propeptide	Gene family	Propeptide size (kDa)	Processed peptide, size	Function	Site of action	Receptor	References
CEP	6	8.5–11.5	CEP1, 14 AA	Inhibits root growth	Lateral root primordia	Unknown	Ohyama et al. (2008)
CLE (and CLV3)	32	7.8–14.5	CLE or mCLV3, 12–14 AA	Stimulates organogenesis and inhibits meristematic growth; can stimulate vascular development	Floral, shoot, and root meristems; vascular	CLV1, BAM1, CLV2, RPK2	Clark et al. (1995, 1997), Cock and McCormack (2001), Fiers et al. (2006), Kondo et al. (2006), DeYoung and Clark (2008), and Kinoshita et al. (2010)
DVL/RTFL	24	4.6–16.2	Not processed	Polarity dependence, cell proliferation, leaf development	Stem, rosette leaves, pedicles, siliques	Unknown	Narita et al. (2004), Wen et al. (2004) and Ikeuchi et al. (2011)
EPF	11	11.5–14.3	Unknown	Promotes epidermal cell division leading to guard cell (stomatal) formation	Epidermis and meristemoid mother cells	TMM, ER, ERL1, ERL2	Hara et al. (2007, 2009) and Hunt and Gray (2009)
IDA and IDL	6	8.4–13	EPIP	Inhibits floral abscission	Abscission zone	HAS, HSL	Butenko et al. (2003) and Stenvik et al. (2006)
PROPEP	7	9.3–12.3	Pep1, 23 AA	Promotes innate immune responses (a danger signal)	Widespread, leaves	Pep1R	Huffaker et al. (2006), Yamaguchi et al. (2006), Pearce et al. (2008), and Qi et al. (2010)
PNP	2	13–14	Unknown	Extracellular, cell expansion, water/ion movement, stomatal opening, inhibits ABA-induced stomatal closure	Leaves, mesophyll and guard cells, root stele, stem	Unknown	Gehring et al. (1996), Maryani et al. (2001), Ludidi et al. (2002), Rafudeen et al. (2003), Wang et al. (2007), Gottig et al. (2008), Ruzvidzo et al. (2011), and Wang et al. (2011)

POLARIS	1	4.6	36 AA	Required for root elongation, lateral root formation, leaf vascular patterning	Embryonic root and seedling	Unknown	Casson et al. (2002) and Chilley et al. (2006)
PSK	6	8.7–9.7	PSK-α, 5 AA	Promotes cell proliferation and longevity, root elongation	Widespread, mesophyll cells, roots	PSKR1	Matsubayashi and Sakagami (1996), Lorbiecke and Sauter (2002), Matsubayashi et al. (2002, 2006), and Kutschmar et al. (2009)
PSY	3	7.9	PSY1, 18 AA	Promotes cellular expansion and proliferation, upregulated by wounding	Mesophyll cells, roots	PSYR1	Amano et al. (2007)
RALF and RALFL	39	7–14	RALF, 25–30 AA	Associated with danger signals, affects growth—inhibits root growth	Widespread in plants	Unknown	Pearce et al. (2001), Silverstein et al. (2007), and Wu et al. (2007)
RGF1	9	13	13 AA	Maintain root stem cell niche in inner layer of columella cells	Root meristem	Unknown	Matsuzaki et al. (2010)
SCRL	27	9.2–11.5	Not processed	Prevents self-fertilization (but not in *A. thaliana*)	Pollen	SRK	Schopfer et al. (1999) and Vanoosthuyse et al. (2001)
TPD	2	19.5	TPD1	Anther development promoting tapetum formation	Anthers	EMS1	Yang et al. (2003) and Jia et al. (2008)

sulfated on its two tyrosine residues [Y(SO_3H)IY(SO_3H)TQ] (Matsubayashi and Sakagami 1996). PSK was first discovered as an essential cell proliferation agent necessary to maintain low-density cell cultures in several species including asparagus, rice, and carrot (Matsubayashi and Sakagami 1996; Matsubayashi et al. 1997; Kobayashi et al. 1999; also see chapters "Peptides Regulating Root Growth, Peptides Regulating Plant Vascular Development, Peptides and the Regulation of Plant Homeostasis, and Processing of Peptides"). It was later found that PSK occurs across the taxa, and now, it is used to promote taxol production in cell cultures (Kim et al. 2006).

This was followed by the identification of a peptide factor from tobacco leaf extracts that induced a rapid alkalinization response in tobacco suspension cells (Pearce et al. 2001). This peptide is a member of a large family known as the Rapid ALkalinization Factors (RALF) or RALF-like (RALFL) found in many plant families with a common highly conserved cysteine-rich motif (CX{4,14}CX{22,51}CX{6,12}CX{5,14}CX{5,6}C) in a relatively divergent polar peptide (Pearce et al. 2001; Silverstein et al. 2007). RALFL peptides are likely to have a ubiquitous role in plants as their sequences have also been found in many plant families (Pearce et al. 2001; Olsen et al. 2002; Haruta and Constabel 2003; Germain et al. 2005; Silverstein et al. 2007; also see chapters "The Role of Plant Peptides in Symbiotic Interactions, Peptides and the Regulation of Plant Homeostasis, and Processing of Peptides"). Since then, several other secreted peptides have been identified from extracellular extracts including peptide 1 (Pep1) (Huffaker et al. 2006), C-terminally encoded peptide 1 (CEP1) (Ohyama et al. 2008), and plant peptide containing sulfated tyrosine (PSY1) (Amano et al. 2007).

Various genetic screens have also resulted in the identification of peptide signals (also see chapter "Methods to Identify New Partners of Plant Signaling Peptides"). These screens are used to reveal components necessary for development, and knockout mutants are selected on the basis of abnormal phenotypic growth patterns. The first peptide to be identified using these screens was CLAVATA 3 (CLV3) where the *clv3* mutant has abnormally large shoot and floral meristems due to excess stem cells (Clark et al. 1995; Fletcher et al. 1999). Mutant studies have the advantage that identifying mutants with similar phenotypes may in turn reveal other components of the signaling pathway that can then be experimentally confirmed. The mutant *clv1* also has abnormally large shoot and floral meristems, and CLV1 encodes a full-length leucine-rich repeat receptor-like kinase (LRR-RLK) (Clark et al. 1997). More recently, it has been shown biochemically that the processed CLV3 peptide directly interacts with the external leucine-rich domain of the CLV1 receptor (Ogawa et al. 2008). The CLE family is named for CLV3 (Clark et al. 1995; Fletcher et al. 1999) from *Arabidopsis* and *EMBRYO SURROUNDING REGION* (*ESR*) from maize (Opshal-Ferstad et al. 1997) and forms one of the largest families of plant peptide signaling molecules that is present throughout the plant kingdom (Cock and McCormack 2001; Oelkers et al. 2008; Sect. 5; chapters "Peptides Regulating Apical Meristem Development, Peptides Regulating Root Growth, and Peptides Regulating Plant Vascular Development," containing 32 annotated genes in *Arabidopsis*. CLE family members contain the CLE domain

(14 amino acids) near the C-terminal (Cock and McCormack 2001), and CLV3 is the best characterized member. Mutant screens that detected abnormal stomatal patterns identified two EPIDERMAL PATTERNING FACTOR (EPF) proteins, EPF1 and EPF2, which are involved in determining epidermal cell division events leading to stomatal formation (Hara et al. 2007, 2009; Hunt and Gray 2009; see chapter "Peptides Modulating Development of Specialized Cells"). More recently, combinations of biochemical and genetic approaches have been used to identify additional peptides regulating plant growth and development such as the root meristem growth factor (RGF) (Matsuzaki et al. 2010, Sect. 9). Another interesting example is the pollen tube attractants (LURE) that have been identified in *Torenia fournieri* (Okuda et al. 2009; Higashiyama 2010), but to date, homologues of these proteins in *Arabidopsis* have not been found, and they are excluded from the phylogenetic analysis in Sect. 5.

An alternative approach based on peptide analogy to animal systems was used to identify and purify a small protein now known as plant natriuretic peptide (PNP) which is immunoreactive to antisera specific for the animal peptide factor, atrial natriuretic peptide (Vesely and Giordano 1991; Gehring et al. 1996; Maryani et al. 2001; Ludidi et al. 2002; see chapter "Peptides and the Regulation of Plant Homeostasis"). The 33-amino-acid active region of PNP-A is the only region with similarity to atrial natriuretic peptide (Wang et al. 2007), and phylogenetic analysis suggests that the similarities between the animal and plant form most likely are the result of convergent evolution (Gehring and Irving 2003).

4 Processing of Peptide Ligands

Once peptide ligands were discovered, it then became evident that they were formed from precursor proteins that had been secreted into the apoplast where the proteins were further processed (Fig. 1). That is, the precursor proteins include an N-terminal signal sequence targeting the proteins for export (preproprotein), and this is cleaved during protein translation to/from the proprotein that is exported by the default secretory pathway (Denecke et al. 1990). Following export, the actual active peptide ligand is proteolytically cleaved from the proprotein. The processes underlying these events are discussed in more detail in chapter "Processing of Peptides." Systemin is the active peptide released from the C-terminal end of the 200-amino-acid precursor protein prosystemin by proteolytic processing systems in the apoplast (McGurl et al. 1992). CLE, PSK, and RALF are also formed from precursor proteins where the final processing into the active peptide occurs in the apoplast, and this is the case for several other peptide molecules as listed in Table 1. Enzymes such as tyrosylprotein sulfotransferase catalyze the sulfation of the tyrosine residues in proPSK as the protein is processed through the Golgi network before being secreted (Hanai et al. 2000). Alternative processing events involve hydroxylation of proline residues which occurs in the CLE motif of the

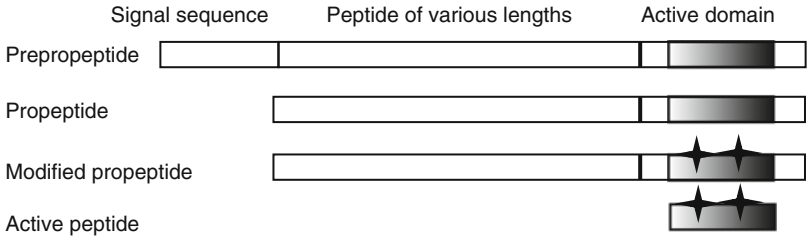

Fig. 1 Processing of peptide signaling molecules. After transcription, the prepropeptide has its secretory signal sequence cleaved in the endoplasmic reticulum forming the propeptide. As the propeptide is processed through the Golgi apparatus, amino acid modifications are made (indicated by *dagger line*) before the propeptide is secreted into the apoplast where the active peptide is released by further proteolytic processing as described in the text. The active domain is generally located in the C-terminal region of the peptide and is indicated by the *darker rectangle* with a dibasic region 5–20-amino-acid residues upstream (*dark line*) that is a proteolytic cleavage point

active mature hydroxyproline CLV3 (Fiers et al. 2006; Kondo et al. 2006) and also CEP1 (Ohyama et al. 2008). Once secreted, further processing occurs in the apoplast, and it is known that specific subtilisin serine proteases cleave dibasic residues upstream from the PSK sequence in proPSK to release an 8-amino-acid fragment (Srivastava et al. 2008). The enzymes necessary to form the final active product PSK are not yet known. However, it is evident that several enzymes and ProPSK need to come together in the apoplast for these processing events to be successful. Presumably for this to occur, the processing enzymes and proproteins are secreted from the same or adjacent cells indicating a large degree of coordinated protein regulation. Equally well-programmed events where the proteolytic enzymes are secreted (or present) in addition to the proprotein are necessary in the processing of RALFL (Srivastava et al. 2009). ProCLV3 is secreted into the meristematic apoplast (Rojo et al. 2002) where it is processed into the active short peptide (Kondo et al. 2006; Ni and Clark 2006). The active small peptides are generally found at the C-terminal of the propeptide molecule, and this region has homology (or is even identical) with other peptides of the same class but not between classes (Sect. 5; Fig. 2).

However, not all proteins appear to be processed into short active peptides. The Low-molecular-weight Cysteine-Rich (LCR or pollen coat protein) and S-locus Cysteine-Rich (SCR) and SCR-like (SCRL) proteins are characterized by eight conserved cysteine residues throughout the relatively small (ranging from 4 to 11 kDa) secreted protein (Vanoosthuyse et al. 2001). The cysteine residues are found throughout the secreted protein and thus contribute to the folding of the SCR or SCRL (see chapter "The S-LOCUS CYSTEINE RICH PROTEIN (SCR): A Small Peptide with a High Impact on the Evolution of Flowering Plants"). The solution structure of an SCR has been resolved, and it folds into an α/β sandwich structure that resembles that of plant defensins with a unique loop bulging out from the body of the protein containing the hypervariable region (Mishima et al. 2003; see chapter "Plant

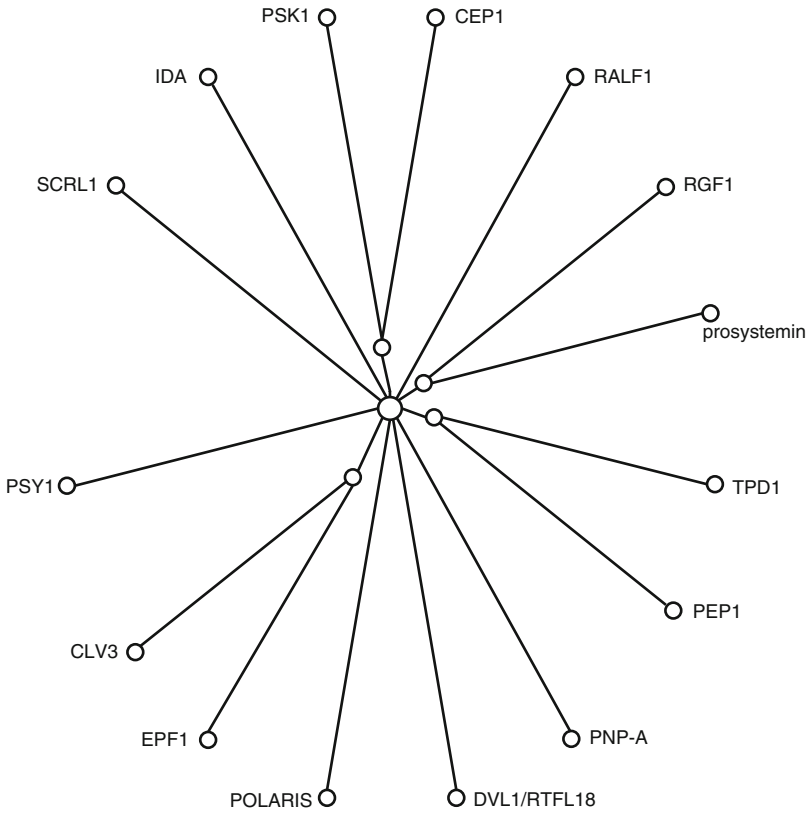

Fig. 2 Phylogenic diagram demonstrating that the different peptide classes are not related. This Radial Cobalt Tree was produced using the National Center for Biotechnology Information (NCBI) COBALT multiple alignment tool. All 175 available *Arabidopsis* amino acid sequences of the different peptide classes as well as the tomato prosystemin segregated into the different peptide classes. In the Radial Cobalt Tree shown, a single-protein sequence was selected to represent each group: CEP (6 sequences), CLE/TDIF (32), DVL/RTFL (24), EPF (11), IDA (6), PROPEP (7), PNP (2), POLARIS (1), PSK (6), PSY (3), RALF (39), RGF (9), SCRL (27), and TPD1 (2). Refer to Table 1 for abbreviations and a description of the known function for each class

Antimicrobial Peptides"). The LURE peptides identified as additional components of the pollen pistil interaction also contain conserved cysteine residues throughout and, like LCRs and SCRs, are defensin-like proteins (Higashiyama 2010; chapter "Plant Antimicrobial Peptides"). The protein PNP-A is secreted from mesophyll cells (Wang et al. 2011), but it is still unknown if PNP is further processed despite some initial speculation (Pharmawati et al. 1998). PNP-A contains six cysteine residues throughout the secreted protein, and the region that contains specific activity occurs toward the N terminus where modeling predicts it to form part of an exposed loop from the β barrel structure (Morse et al. 2004; Wang et al. 2007).

5 Phylogenic Relationships Between Classes of Peptide Ligands

Of the small secreted peptides identified to date, most have representatives in agronomically important monocot and dicot lineages including rice (*Oryza sativa* L.), maize (*Zea mays* L.), sorghum [*Sorghum bicolor* (L.) Moench], soybean [*Glycine max* (L.) Merr.], castor oil bean (*Ricinus communis* L.), wine grape (*Vitis vinifera* L.), and the black cotton wood tree [*Populus balsamifera* L. Ssp. *trichocarpa* (Torr. and A. Gray ex hook) Brayshaw]. Some peptide groups such as EPF and CLE can be found across the plant kingdom including moss (*Physcomitrella patens*) and lycophytes (*Selaginella moellendorffii*) (Miwa et al. 2009; Floyd and Bowman 2010; Rychel et al. 2010). Examples of the CLE family have also been found in green algae (*Chlamydomonas reinhardtii*) (Oelkers et al. 2008) and the nematode *Heterodera glycines* (Olsen and Skriver 2003) where it is believed to be an example of horizontal gene transfer. The CLE peptide secreted by the nematode is thought to mimic the endogenous CLE in soybean roots (Jun et al. 2008; see chapter "The Role of Plant Peptides in Symbiotic Interactions"). PNP (sometimes annotated as expansin-like) also occurs widely across the plant kingdom (Ludidi et al. 2002). A unique analogue of PNP has been reported in the bacterial pathogen *Xanthomonas axonopodis* pv *citri* that can alter host plant homeostasis mechanisms, and it is thought that this is another example of horizontal gene transfer (Nembaware et al. 2004; Gottig et al. 2008; see chapter "Peptides and the Regulation of Plant Homeostasis"). Most other peptide groups listed in Table 1 such as CEP, DEVIL/ROTUNDIFOLIA4 (DVL/RTFL originally called DVL1/ROT4), INFLORESCENCE DEFICIENT IN ABSCISSION (IDA), Pep1, PSK, RALF, and TAPETUM DETERMINANT (TPD1) are represented in angiosperms including both monocots and dicots (Matsubayashi and Sakagami 1996; Matsubayashi et al. 1997; Pearce et al. 2001; Butenko et al. 2003; Yang et al. 2003; Narita et al. 2004; Wen et al. 2004; Huffaker et al. 2006; Silverstein et al. 2007; Combier et al. 2008; Ohyama et al. 2008; Zhao et al. 2008). A smaller number are represented only in dicots including two sulfated peptide families, PSY and the recently identified RGFs (Amano et al. 2007; Matsuzaki et al. 2010). Finally, some peptide groups are further limited to a single family such as systemin which is restricted to Solanaceae (Ryan and Pearce 1998) and SCRL to the Brassicaceae (Schopfer et al. 1999; Vanoosthuyse et al. 2001) while POLARIS is found only in *Arabidopsis* (Casson et al. 2002).

Of the 15 peptide groups examined, all but systemin have representatives in the model plant *A. thaliana*. Comparison of all 175 available *Arabidopsis* peptide sequences, along with tomato systemin, shows that peptides of the same group are more similar to each other than any peptide from another group (with the exception of RGF5 and RGF8 which are most similar to CLE46 and CLE45, respectively). This is despite the low level of homology between peptides of the same group outside the defining peptide domain. A single-protein sequence was

selected to represent each peptide group, and a radial COBALT tree shows the peptides in relation to one another (Fig. 2) where, for example, CLV3 was chosen to represent the CLE family. The number of members of the peptide groups we examined varied from 1 or 2 (POLARIS and TPD) to 39 (RALFL), and groups with many members (CLE 32, SCRL 27, and DVL/RTFL 24) did not necessarily correlate with ancient lineages. An EPF sequence homologue has been found in moss while the prolific SCRL family has 27 members in *Arabidopsis* but is limited to the Brassicaceae (Schopfer et al. 1999; Vanoosthuyse et al. 2001).

Within each peptide group, the members were often spread across the chromosomes, but in some cases, the peptide-encoding genes were clustered. For example, six genes of proPep are clustered in two groups on chromosome 5 and another gene (proPep6) occurs on chromosome 2, but it is more similar to members of one of the clusters than the other (Wheeler and Irving 2010). Similarly, the more expanded signaling peptide groups such as RALFL, SCRL, and CLE are also clustered. In the case of RALFL, several encoding gene groups are clustered similarly on both the *Arabidopsis* and rice chromosomes (Silverstein et al. 2007) and examples include those most similar in sequence such as RALFL2 and 3, RALF8 and 9, RALF10–13, and RALF25 and 26. However, it should be noted that close proximity of encoding genes does not necessarily mean there is a high level of sequence similarity. Interestingly, in the IDA group of peptides, the two most closely related genes IDL2 (At5g64667) and IDL3 (At5g09805) are found in duplicated regions of chromosome 5, covering genes At5g63600–65640 and At5g08570–10570 although neither IDL2 nor IDL3 is sufficient to complement IDA, implying that these gene products are not redundant (Stenvik et al. 2008).

In fact, in some peptide families such as EPF, the functional differences among family members appear to be defined by their distinct spatiotemporal expression patterns and this is the case for EPF1 and EPF2. These genes are expressed at late and early stages of stomatal lineage progression respectively and display loss of function phenotypes consistent with their time of expression (Abrash and Bergmann 2010; Abrash and Lampard 2010; Peterson et al. 2010; Rychel et al. 2010; chapter "Peptides Modulating Development of Specialized Cells"). Differential expression of genes from the same peptide group encoding the same processed signal peptide can occur as is the case with the PSK family (Yang et al. 2001; Matsubayashi et al. 2006). It has also been shown that in rice, *Medicago*, wheat (*Triticum aestivum*), and *Selaginella,* a single CLE gene encodes multiple CLE domains (Cock and McCormack 2001; Kinoshita et al. 2007; Miwa et al. 2009). In rice, CLE75 encodes 6 CLE domains, and in *Selaginella,* SmCLE15 has 8 CLE domains encoding two different classes of CLE peptides. It is suggested that multiple peptides produced from one precursor will contribute to a rapid response and that these events may be specific to rice, *Medicago*, wheat, and *Selaginella* as they are not found in *Arabidopsis* (Kinoshita et al. 2007; Miwa et al. 2009).

Interestingly, BLAST analysis of all the *Arabidopsis* peptides from the RGF peptide group shows that each peptide is more similar to orthologs found in other species than to the other *Arabidopsis* RGF peptides. For instance, RGF6 (At4g16515) has higher homology with *Arabidopsis lyrata* (XP_002868128), castor oil bean (XP_002525906), black cotton wood tree (XP_002329915, XP_002329923), and table grape (CAN75750, CBI31912.3). Comparison of more abundantly spread peptide sequences over a range of species using PROPEP and PSK has shown that sequence similarity separates along monocot and dicot lineages (Lorbiecke et al. 2005; Huffaker et al. 2006).

6 Paracrine and Autocrine Effects

A theme that will become evident in this book is that many plant signaling peptides are expressed in specific and often restricted regions of the plant where they are secreted, further processed, and act upon nearby cells (Fig. 3). This action is similar to growth factors and cytokines regulating development in animal cells where paracrine and autocrine effects are paramount (Ozaki and Leonard 2002). This type of signaling is very ancient and evident throughout multicellular organisms and is probably based on signaling mechanisms developed in single-cell organisms such as quorum sensing in bacteria (e.g., Hibbing et al. 2010). It has the major advantage of allowing a gradient of molecules to form from the secretory to recipient cells which creates a tailored signaling microenvironment that can stimulate different responses in the nearby cells depending on the strength of the signal. The ligands involved in this type of gradient signaling are sometimes referred to as morphogens. Most of the peptides listed in Table 1 exhibit paracrine and/or autocrine effects acting within specific regions such as floral or shoot meristems as shown in Fig. 3. This is particularly marked with peptides directly affecting development such as CLE, EFP, IDA, and RGF which act at specific localized regions within the plant (see chapters "Peptides Regulating Apical Meristem Development, Peptides Regulating Root Growth, Peptides Regulating Plant Vascular Development, Peptides Modulating Development of Specialized Cells, and The Role of Plant Peptides in Symbiotic Interactions").

7 Peptide Ligand Receptors

For cells to respond to the peptide ligands, receptors have to be present, and it is thought that receptors and their ligands have evolved in parallel (Fryxell 1996). Receptors for the peptide ligands that have been identified are generally members of receptor-like protein kinase families such as the leucine-rich repeat receptor-like kinases (LRR-RLKs), and several recent reviews have discussed the different types

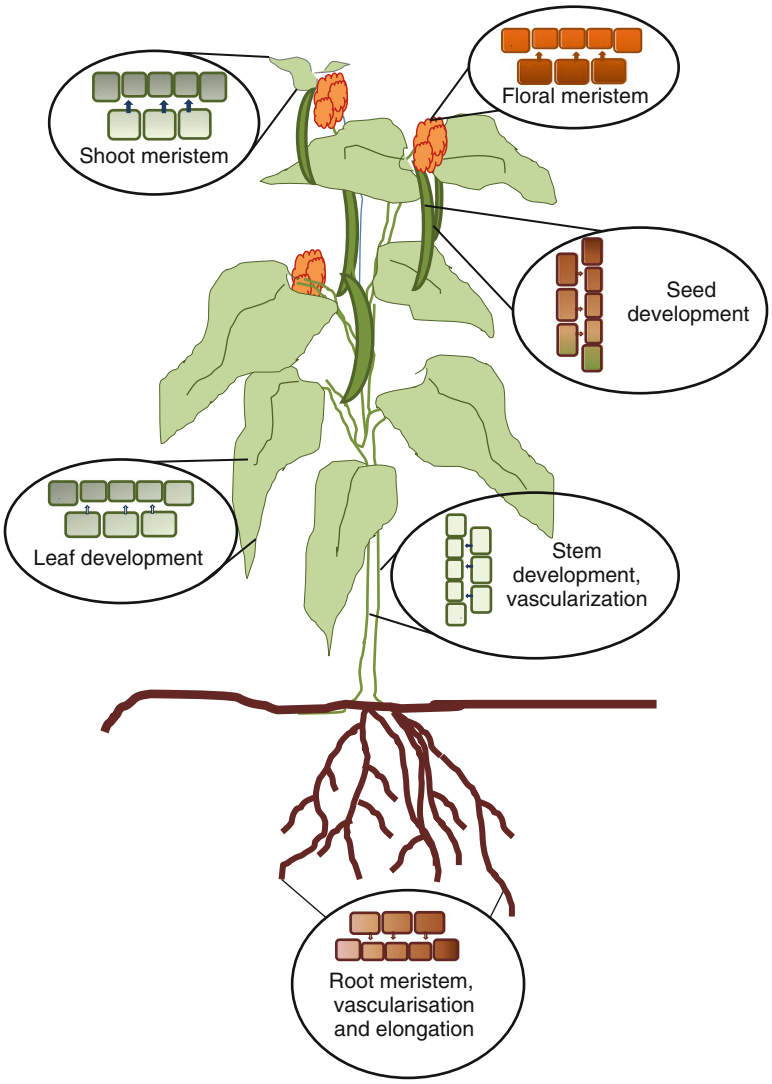

Fig. 3 Paracrine and restricted regions for peptide signaling. In many cases, peptide signaling molecules are released from a localized group of cells into the apoplast where they form a concentration gradient that most strongly acts on nearby cells containing receptors. Peptides acting locally (paracrine) are known to affect cell development in the meristems (floral, shoot, and root), seeds, vasculature, and formation of guard cells in leaves as shown

of receptors and the signaling mechanisms activated following binding of peptides or other ligands (see, e.g., Atzal et al. 2008; Boller and Felix 2009; Tör et al. 2009; Clouse 2011). The receptors for peptides can form oligomers and often exist as either homomeric or heteromeric dimers (Fig. 4). The extracellular regions of the receptors recognize particular surface patterns of the active peptide ligands that are

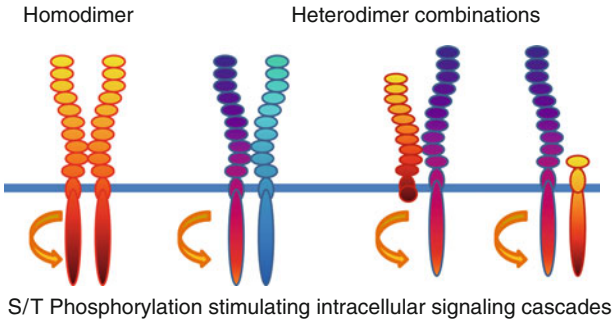

Fig. 4 Models of functional receptor oligomers that can form as homo- or heterodimers. In each case, a component will be an LRR-RLK which contains a leucine-rich repeat external domain, a single transmembrane-spanning domain, and an intracellular (cytoplasmic) kinase domain. Oligomers form between the same or different LRR-RLKs where both receptor proteins contain all of the functional domains. Alternatively, oligomers can form with one LRR-RLK and either an LRR receptor-like protein that contains no kinase domain or membrane-associated kinase protein containing a limited extracellular domain. Upon ligand binding and oligomer formation, the kinase domains autophosphorylate serine and threonine residues and initiate an intracellular phosphorylation cascade

present in the extracellular matrix. In some cases, a receptor may recognize more than one peptide perhaps due to the common surface pattern formed by the peptide. This is likely to be the case with the CLE peptides. There are 32 known CLE peptides in *Arabidopsis* which all contain a common CLE motif but are derived from different precursor proteins with specific regional expression patterns. Hence, the effects of the peptide ligands are limited to areas where the peptides are actually secreted and processed, and this has been shown with CLE ligands where expression of the receptors involved in organogenesis is restricted to localized areas (Müller et al. 2008). When CLE peptides are applied exogenously, it results in a gradient of responses, and different members can mimic each other (Whitford et al. 2008), which is indicative of overlapping redundancy in the receptor specificity for small but highly similar active peptide fragments. CLV3 was the first of these ligands discovered and it directly interacts with the extracellular domain of CLV1 which is a full-length LRR-RLK (Ogawa et al. 2008), and CLV1 has been shown to form heterodimers with BARELY ANY MERISTEM 1 (BAM1) which is a related LRR-RLK (DeYoung and Clark 2008). More recently, another LRR-RLK, homomers of receptor-like PROTEIN KINASE 2 [RPK2, also known as TOADSTOOL 2 (TOAD2)], has been shown to interact with CLV3 (Kinoshita et al. 2010).

The interaction between ligand and receptor has not been as well characterized for the other signaling peptides. Binding and cross-linking affinity studies were used to identify receptors for PSK, PSY1, and Pep1, and these receptors are all members of the LRR-RLK family (Matsubayashi et al. 2002, 2006; Yamaguchi et al. 2006; Amano et al. 2007; Jia et al. 2008, chapter "Methods to Identify New Partners of Plant Signaling Peptides"). LRR-RLK proteins contain a large extracellular leucine-rich repeat domain that has a specific peptide-recognizing region

within it, a single transmembrane-spanning region, and an intracellular kinase domain thought to mediate a phosphorylation signaling cascade. Several of the peptide receptors [PSKR1, PepR1, CLV1, and ERECTA (ER)] also contain a putative guanylate cyclase catalytic center within the intracellular general kinase domain region (Kwezi et al. 2007, 2011; Qi et al. 2010), and this has led to speculation that production of cGMP may form part of the signaling pathway in addition to phosphorylation cascades. Indeed, AtPSKR1 does demonstrate some guanylate cyclase activity in vitro while PSK-α stimulates cGMP production in protoplasts (Kwezi et al. 2011).

8 Cost–Benefits of Peptide Ligands

Peptide ligands are relatively nitrogen rich (amino acids contain one to four N atoms) and, in some cases, also either sulfated due to cysteine (or methionine residues) or posttranslationally sulfated [e.g., phytosulfokines (Hanai et al. 2000)]. Both nitrogen and sulfur are nutrients that limit plant growth (Hawkesford and De Kok 2006; Elser et al. 2007), so it would appear counterintuitive for plants to invest in nitrogen-rich signaling molecules. However, it has recently been shown that ecological nitrogen limitations have influenced both the transcribed RNA and proteome nitrogen content such that crop and nitrogen-fixing plants contain higher nitrogen levels in their transcribed RNA and more amino acids with nitrogen-rich side chains than undomesticated plants (Acquisti et al. 2009). We hypothesized that the relative importance of the plant peptides to the plants may be reflected in the nitrogen level of the precursor proteins (Wheeler and Irving 2010). We observed that prepropeptide molecules collectively had higher levels of nitrogen-containing amino acids than the average across all proteins in *Arabidopsis*. This finding led us to argue that plants could efficiently use the limited nitrogen and sulfur resources by restricting expression of these peptides to areas such as the meristem (Wheeler and Irving 2010). An advantage in using peptides as the signaling molecules also lies in the fact that they can be easily upregulated in specific cells by gene transcription in response to environmental triggers. Whether this is a more efficient process than generating the classical hormones is open to question. However, a gradient signal will be produced that can act in a local (paracrine) environment over a few hours reflecting the constraints of gene transcription. More rapid responses can also occur if partially processed peptides are secreted from intracellular stores and/or further processed in the apoplast (extracellular matrix) by various proteolytic enzymes to generate the signaling peptide (Srivastava et al. 2008, 2009; chapter "Processing of Peptides"). The propeptide is relatively inactive compared with the cleaved mature active peptides which are active at nanomolar or lower concentrations (Matsubayashi and Sakagami 1996; Ito et al. 2006; Pearce et al. 2008). Thus, the presence of the additional processing enzymes in the apoplast provides a further level of control in determining the level of activity of peptide signaling molecules.

9 Mix and Match Signaling

Since the receptors for peptides form as oligomers composed of one or two (or more) separate subunits, a degree of selection is introduced to perception of the peptide ligand by receptors in various regions within the plant. In addition, different peptides can act to counteract the effect of another peptide and so bring in a degree of control at the level of perception (also see chapters "Peptides Regulating Plant Vascular Development and Peptides Modulating Development of Specialized Cells"). A further degree of adaptability is introduced by the fact that, in many cases, multiple proteins are precursors for very similar peptide ligands and these are expressed in quite particular and restricted regions of the plant. Even though this results in considerable redundancy in the effects of the peptide class, the restricted spatiotemporal patterns of expression act to counterbalance this redundancy. Here, we will use two examples to explore this concept using the CLE and RGF peptide families.

Most members of the CLE family are known as CLE A, and these act to repress cell division in the meristematic regions while the CLE B (specifically CLE41–44) influence development of cells forming the vascular system (Whitford et al. 2008). The CLE B family members are homologues of the *Zinnia elegans* tracheary element differentiation factor that suppresses xylem cell differentiation in cultured mesophyll cells (Ito et al. 2006). In addition, PSK-α appears to act in a cooperative manner with CLE B peptides. PSK-α promotes tracheary element differentiation in *Zinnia* mesophyll cell cultures in the presence of auxin and cytokinin (Matsubayashi et al. 1999; Motose et al. 2009), whereas the CLE B peptides inhibit this process (Ito et al. 2006; for a discussion, see Fukuda et al. 2007; and chapter "Peptides Regulating Plant Vascular Development"). PSK-α has a general proliferative effect and was discovered as a cell proliferation agent essential for low-density cell cultures (Matsubayashi and Sakagami 1996; Matsubayashi et al. 2006). Investigations into the CLE A family have revealed that the different members can have overlapping effects that are not normally seen due to spatial or temporal separation of their expression. For instance, CLE19 is normally found in roots, and ectopic application of synthetic peptides corresponding to the overlapping conserved CLE motifs of CLV3, CLE19, and CLE40 caused the termination of the root meristem, resulting in a similar phenotype to overexpressed CLE19 mutants (Fiers et al. 2006). These results indicate that the receptor recognizes the overlapping CLE motif but not specific CLE peptides. The receptor for CLV3 is likely to be an oligomer of the LRR-RLKs CLV1 and BAM (DeYoung and Clark 2008; Ogawa et al. 2008) or homomers of RPK2 (Kinoshita et al. 2010), but whether these receptor combinations are expressed in roots and responsive to CLE19 remains to be determined (also see chapter "Peptides Regulating Root Growth"). In addition, applications of combinations of CLE A and B peptides result in favoring proliferation of vascular development indicating that the CLE B peptides are dominating the development in this instance (Whitford et al. 2008). This suggests that a reciprocal gradient will form CLE A and B type peptides in the

meristem, and this, in turn, will regulate organogenesis and vascular development. This effect is likely to be due to expression of the same or similar classes of receptors recognizing different combinations of CLE ligands—a mix and match combination of ligand and receptor. Such events are probably not surprising as the CLE ligand is relatively conserved (Cock and McCormack 2001), and it is likely that multiple combinations of CLE receptors are expressed in the developing vascular and meristematic regions (also see Fukuda et al. 2007; Jun et al. 2008; chapters "Peptides Regulating Plant Vascular Development and The Role of Plant Peptides in Symbiotic Interactions").

Another example of mix and match signaling involves the recently identified tyrosine-sulfated peptide family RGF which contains nine members in *Arabidopsis*. The mature active peptides are 13 amino acids long with a sulfated tyrosine residue and are necessary to maintain the root meristem (Matsuzaki et al. 2010). The RGF peptides were discovered by database searches after it was observed that the tyrosylprotein sulfotransferase (TPST) knockout mutant *tpst* had abnormally small root meristems that could not be rescued by application of PSK or PSY (the other two known sulfated peptides). Application of synthetic RGF1–6 clearly rescues the *tpst* phenotype, whereas RGF7 and 9 have weak activity and RGF8 has no activity. RGF acts posttranscriptionally to define the expression levels and patterns of the AP2 domain transcription factor plethora2 (PLT2), creating a gradient in the stem cell niche that has been shown using ectopic application with RGF attached to dextran beads (Matsuzaki et al. 2010). Such findings highlight the importance of both spatial and temporal differentiation in the expression patterns of the CLE and RGF (and other) peptides to prevent developmental errors.

10 Conclusions

Peptide signaling molecules represent an ancient evolutionary adaptation that is used by all organisms as part of the rapid and modular responsive system to environmental challenges and to regulate growth and development. Although peptide signals have only been identified in plants in the last two decades or so, it is now apparent that plants contain a diverse group of peptide signaling molecules that have independent lineages. Some of these peptide families contain numerous members that participate in mix and match modular signaling with receptor combinations, and this will provide the plant with flexibility in regulating responses to the peptides. In many cases, paracrine signaling appears to be an important aspect where the degree of response is modulated by a gradient which ensures that particular regions such as meristems respond to the signal. A further advantage that may be associated with peptide signaling is that relatively rapid and controlled release can be achieved by not only secreting the prepropeptide but also processing enzymes that ensure that the mature peptide is released. From a nitrogen and energy use perspective at least, it may be relatively cheap for plants to use peptide signals as often there are only a few cells making this demand on nitrogen and energy

resources. It is likely that even more peptide signaling molecules will be discovered as many small peptides are not annotated in the databases (Silverstein et al. 2007), and this is attested by the recent discoveries of RGFs (Matsuzaki et al. 2010) and LUREs (Higashiyama 2010). At this stage, only a few receptor–ligand pairs are known, and further receptors are likely to be identified for the peptide ligands (see Butenko et al. 2009; chapter "Methods to Identify New Partners of Plant Signaling Peptides"). In the final section of this book, methods to study and discover peptide signaling molecules are examined in detail (chapters "Methods to Isolate and Identify New Plant Signaling Peptides, Methods to Identify New Partners of Plant Signaling Peptides, and Computational-Based Analysis to Associate the Function of Plant Signaling Peptides with Distinct Biological Processes").

Acknowledgments This work was supported by the Australian Research Council's Discovery project funding scheme (DP0557561, DP0878194).

References

Abrash EB, Bergmann DC (2010) Regional specification of stomatal production by the putative ligand CHALLAH. Development 137:447–455

Abrash EB, Lampard GR (2010) A view from the top: new ligands controlling stomatal development in Arabidopsis. New Phytol 186:561–564

Acquisti C, Elser JJ, Kumar S (2009) Ecological nitrogen limitation shapes the DNA composition of plant genomes. Mol Biol Evol 26:953–956

Amano Y, Tsubouchi H, Shinohara H, Ogawa M, Matsubayashi Y (2007) Tyrosine-sulfated glycopeptide involved in cellular proliferation and expansion in *Arabidopsis*. Proc Natl Acad Sci U S A 104:18333–18338

Antunes LCM, Ferreira RBR, Buckner MMC, Finlay BB (2010) Quorum sensing in bacterial virulence. Microbiology 156:2271–2282

Atzal AJ, Wood AJ, Lightfoot DA (2008) Plant receptor-like serine threonine kinases: roles in signalling and plant defense. Mol Plant Microbe Interact 21:507–517

Banting FG, Best CH, Collip JB, Macleod JJR, Noble EC (1922a) The effects of insulin on experimental hyperglycaemia in rabbits. Am J Physiol 62:559–580

Banting FG, Best CH, Collip JB, Macleod JJR, Noble EC (1922b) The effect of pancretic extract (insulin) on normal rabbits. Am J Physiol 62:162–176

Bardwell L (2004) A walk-through of the yeast mating pheromone response pathway. Peptides 25:1465–1476

Boller T, Felix G (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu Rev Plant Biol 60:379–406

Butenko MA, Patterson SE, Grini PE, Stenvik G-E, Amundsen SS, Mandal A, Aalen RB (2003) *Inflorescence deficient in abscission* controls floral organ abscission in Arabidopsis and identifies a novel family of putative ligands in plants. Plant Cell 15:2296–2307

Butenko MA, Vie AK, Brembu T, Aalen RB, Bones AM (2009) Plant peptides in signalling: looking for new partners. Trends Plant Sci 14:255–263

Casson SA, Chilley PM, Topping JF, Evans M, Souter MA, Lindsey K (2002) The *POLARIS* gene of Arabidopsis encodes a predicted peptide required for correct root growth and leaf vascular patterning. Plant Cell 14:1705–1721