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Article Addendum

Peptide signals for plant defense display a more universal role

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Hydroxyproline-rich systemins (HypSys) are small defense signaling glycopeptides found within the Solanaceae family that until recently were thought to only induce defense genes to herbivore attack. The glycopeptides are processed from larger proproteins with up to 3 different glycopeptides being processed out of a single precursor protein. A conserved central hydroxyproline motif within each HypSys is the site of pentose sugar attachment. Recently, it was found that in *Petunia hybrida*, these defense signaling glycopeptides did not induce protease inhibitor but instead, increased levels of *defensin*, a gene that is involved in pathogen attack. More recently, a HypSys peptide was isolated from *Ipomoea batatas* (sweet potato) of the Convolvulaceae family and found to induce *sporamin*. The proprotein precursor contained six putative peptide signals and had a propeptidase processing region with homology to solanaceous proHypSys. Thus, the HypSys defense peptides are no longer confined to defense against herbivory or exclusivity to the Solanaceae family, redefining both function and dispersion.

Plants have evolved an arsenal of defense mechanisms for survival against the wide array of predators and pathogens that they encounter. Each species has evolved within its unique environment and the protective defense mechanisms must evolve and refine over time to allow a plant to compete in its niche.[1] Plant peptide signals have recently been discovered that induce defense genes for protection against both herbivores and pathogens.[2] This raises the issue of how these peptides, their receptors, signaling pathways, and the downstream regulated defense proteins and compounds have evolved to meet the unique and specific needs of each plant. Our recent papers[3,4] reveal that these defense signaling peptides are not confined to a single family of plants and that the end products of the signaling pathway may be more diverse than expected.

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Systemin was the first peptide signal discovered in plants.[5] The 18 amino acid peptide is processed from the C-terminal of a 200 amino acid precursor; prosystemin.[6] Although lacking a signal sequence, prosystemin reaches the apoplast and the mature peptide is processed upon insect attack, signaling downstream events leading to the production of defense proteins, such as polyphenol oxidase and protease inhibitors.[7] Systemin has only been found in the Solanaceae family and more specifically, only in the subfamily Solanoideae, which contains tomato, potato, nightshade and pepper.

The hydroxyproline-rich systemin glycopeptides are similar to systemin in size (18–20 amino acids in length) and, like systemin, are processed from larger precursors.[2,8] Both systemin and HypSys induce the production of methyl jasmonate and function to amplify the defense response. Each HypSys peptide contains a hydroxyproline-rich inner core that is the site of glycosylation and both the peptide backbone and the carbohydrate moieties are important for receptor recognition (Table 1). The HypSys precursors, unlike prosystemin, contain a signal sequence, which, along with the post-translational modifications, indicate that they are secreted to the apoplast. Both systemin and HypSys have been localized to the plant vasculature.[9,10] Although there is no sequence similarity between prosystemin and hydroxyproline-rich systemins, it has been suggested that because of their size, structure and functional similarities, they should be classified together.[11]

A second defense peptide family, the *At*Peps, was recently discovered in Arabidopsis and like systemin, the precursors lack a signal sequence but the mature peptide interacts with the extracellular domain of a membrane bound receptor.[3,12] The active peptides are 23 amino acids in length and like systemin, processed from the extreme C-terminus. One of the major induced defense genes of the *At*Peps is *defensin* and the *At*Peps have been found to protect the plant from pathogen attack.[12] *At*Pep orthologs have been found in many of the major crop plants.

The precursors for HypSys peptides, unlike prosystemin, were found in a wider range of Solanaceous plants including the Cestroideae subfamily that includes tobacco and petunia. Each precursor contained multiple peptide signals; for instance, tobacco contained 2 HypSys peptides per precursor,[13] tomato with 3 HypSys peptides,[14] nightshade with 3 HypSys peptides,[15] potato with 3 HypSys peptides,[16] and most recently petunia with 3 and possibly 4 HypSys peptides per precursor.[5] Surprisingly, the petunia HypSys peptides were found to induce the pathogen defense gene, *defensin*, like the *At*Peps, rather than proteinase inhibitors. This expands

the known role of HypSys peptides from exclusive involvement in protection from herbivory to broader defense responses, including pathogen defense.

A second major finding was the isolation of the first non-solanaceous HypSys peptide from sweet potato, a member of the Convolvulaceae family.[4] The precursor was larger than any found within the Solanaceae (291 amino acids in length), and contained a surprising 6 putative signaling peptides. The precursor contained a signal sequence and a propeptidase splicing region with homology to the Solanaceae precursors. Since the discovery of the sweet potato proHypSys, candidate proHypSys genes have been found in nucleotide data bases of other non-solanaceous plants, including poplar and coffee (Table 1). Cumulatively, these findings indicate that the HypSys peptide defense system may be utilized by a wide variety of plants and may elicit a defense response against either herbivores or pathogens or possibly both.

These recent findings have revealed two peptide signaling systems that work through a receptor mediated cascade to produce defense proteins. Future work will elucidate which peptide signal is working in which plants, whether HypSys and *At*Pep homologs might work together within the same plant, and whether there are other plant defense peptide systems yet to be discovered.

Table 1 Comparisons of the amino acid sequences of isolated and putative Systemin and HypSys peptides

Peptides	Peptide Sequences	Pentose Units
Sl systemin*	AVQSKPPSKRDPPKMQTD	
St systemin I	AVHSTPPSKRDPPKMQTD	
St systemin II	AAHSTPPSKRDPPKMQTD	
Sn systemin	AVRSTPPPKRDPPKMQTD	
Ca systemin	AVHSTPPSKRPPPKMQTD	
*Nt*HypSys I*	RGANLPOOSOASSOOSKE	9
*Nt*HypSys II*	NRKPLSOOSOKPADGQRP	6
*Sl*HypSys I*	RTOYKTOOOOTSSSOTHQ	8-17
*Sl*HypSys II*	GRHDYVASOOOOKPQDEQRQ	12-16
*Sl*HypSys III*	GRHDSVLPOOSOKTDPIIGQ	6
StHypSys I	RTPYITPTPPTSSPPTNQE	
*St*HypSys II	GRHDHVAAPPPPEPQDEQRQ	
*St*HypSys III	GRHDHVLPPPSPKHEPIIGQ	
*Ph*HypSys I*	RSLHKSOOOTOKPSDEQGQ	10
*Ph*HypSys II*	RHDYHLPOOOAOKPADHTGQ	10
*Ph*HypSys III*	RGKRLPOOAOEYDPOYHQ	3-6
*Sn*HypSys I*	GRHDHVOAOOAOKPEDEQGQ	6
*Sn*HypSys II*	RNRPYITOSOOEASOSTKQ	6
*Sn*HypSys III*	GRHDHVLPOOSOKHEPIIGQ	6
*Ib*HypSys I	REAKSPPPSPKPSDPKNP	
*Ib*HypSys II	RGAKSPPPSPKPSDPINP	
*Ib*HypSys III	REPKSPPPSPKPSDPKNP	
*Ib*HypSys IV*	REEKPOOOAOETDDPNRP	6-12
*Ib*HypSys V	REARSPPPAPEKDIPTHP	
*Ib*HypSys VI	RTARPPPPAPKPAAPIHP	
*Pt*HypSys I	GRSYSSPPPSPSPIPSPSSE	
*Pt*HypSys II	GRTTPSPPPPKPASPKGELE	
*Cc*HypSys I	GRPLSAPPPPMPASPTHYHE	
*Cc*HypSys II	GRPLSAPPPPMPASPTHYHE	
*Cc*HypSys III	GRPLSPPPSPKPASPTHYHE	

Tomato systemin was aligned with the putative homologs from potato (*St* systemin I and II, *Solanum tuberosum*), nightshade (*Sn* systemin, *Solanum nigrum*), and pepper systemin (*Ca* systemin, *Capsicum annuum*). HypSys peptide from tobacco (*Nt*HypSys I and II), tomato (*Sl*HypSys I, II and III), petunia (*Ph*HypSys I, II and III, *Petunia hybrida*), nightshade (*Sn*HypSys I, II and III), and sweet potato (*Ib*HypSys IV, *Ipomoea batatas*), and the putative peptides encoded in the precursor protein deduced from the cDNA of sweet potato (*Ib*HypSys I, II, III, V, and VI) were aligned by the hydroxyproline/proline central motif. The poplar (*Pt*HypSys I and II, *Populus trichocarpa*) sequences were deduced from Map Viewer Gnomon model: hmm3236034, and the coffee (*Cc*HypSys I, II and III, *Coffea canephora*) sequences were deduced from Unigene SGN-U311058 in the Sol Genomics Network (http://sgn.cornell.edu). The hydroxyproline-rich regions of the isolated peptides are red and the proline-rich regions of the systemins and the putative HypSys peptides are blue. The isolated peptides are marked with a star.

References

1. Walling LL. The myriad plant responses to herbivores. J Plant Growth Reg 2000; 19:195-216.
2. Narvaez-Vasquez J, Orozco-Cardenas ML. Systemins and *At*Peps: Defense-related peptide signals. In: Schaller A, ed. Induced plant resistance to herbivory. Springer Science 2008; 313-28.
3. Pearce G, Siems WF, Bhattacharya R, Chen Y-C, Ryan CA. Three hydroxyproline-rich glycopeptides derived from a single petunia polyprotein precursor activate defensin *I*, a pathogen defense response gene. J Biol Chem 2007; 282:17777-84.
4. Chen Y-C, Siems WF, Pearce G, Ryan CA. Six peptide wound signals derived from a single precursor protein in *Ipomoea batatas* leaves activate the expression of the defense gene *sporamin*. J Biol Chem 2008; 283:11469-76; DOI: 10.1074/jbc.M709002200.
5. Pearce G, Strydom D, Johnson S, Ryan CA. A polypeptide from tomato leaves activates the expression of proteinase inhibitor genes. Science 1991; 253:895-7.
6. McGurl B, Pearce G, Orozco-Cardenas M, Ryan CA. Structure, expression and antisense inhibition of the systemin precursor gene. Science 1992; 255:1570-3.
7. Bergey D, Howe G, Ryan CA. Polypeptide signaling for plant defensive genes exhibits analogies to defense signaling in animals. Proc Natl Acad Sci USA 1996; 93:12053-8.
8. Ryan CA, Pearce G, Moura D, Scheer J. Polypeptide hormones. Plant Cell 2002; 14:251-64.
9. Narváez-Vásquez J, Ryan CA. The cellular localization of prosystemin: a functional role for phloem parenchyma in systemic wound signaling. Planta 2004; 218:360-9.
10. Narváez-Vásquez J, Pearce G, Ryan CA. The plant cell wall matrix harbors a precursor of defense signaling peptides. Proc Natl Acad Sci USA 2005; 102:12974-7.
11. Pearce G, Narvaez-Vasquez J, Ryan CA. Systemins. In: Kastin AJ, ed. Handbook of Biologically Active Peptides. Academic Press 2006; 49-54.
12. Huffaker A, Pearce G, Ryan CA. An endogenous peptide signal in Arabidopsis activates components of the innate immune response. Proc Natl Acad Sci USA 2006; 103:10098-103.
13. Yamaguchi Y, Pearce G, Ryan CA. The cell surface leucine-rich repeat receptor for *At*Pep1, an endogenous elicitor in Arabidopsis, is functional in transgenic tobacco cells. Proc Natl Acad Sci USA 2006; 103:10104-9.
14. Pearce G, Moura DS, Stratmann J, Ryan CA. Production of multiple plant hormones from a single polyprotein precursor. Nature 2001; 411:817-20.
15. Pearce G, Ryan CA. Systemic signaling in tomato plants for defense against herbivores: Isolation and characterization of three novel defense-signaling glycopeptide hormones coded in a single precursor gene. J Biol Chem 2003; 278:30044-50.
16. Pearce G, Bhattacharya R, Chen Y-C, Siems WF, Ryan CA. manuscript in preparation 2008; GenBank Ascession #EU482410.
17. Bhattacharya R, Pearce G, Ryan CA. 2008; GenBank Ascession #EU482409.