

Peptide signaling in plants

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In plants, only a few peptides have been identified that act as signaling molecules (1). In contrast, signaling peptides are major players in all aspects of the life cycle in animals and yeast (2), suggesting that signaling mechanisms across the eukaryotic kingdom are fundamentally different. In animals and yeast, signaling peptides are synthesized, in general, as part of longer biologically inactive preproteins, from which they are released by specific proteases. The few signaling peptides studied in plants so far indicate that plants do not follow a similar approach to generate peptides. In a recent issue of PNAS, however, a tobacco polypeptide is described, showing the typical features of a stereotypical preproprotein from which an active signaling peptide, called rapid alkalization factor (RALF), is released (3).

RALF is a 5-kDa peptide that is located at the C terminus of a 115-aa long preproprotein. The peptide was identified in a bioassay involving a suspension culture of tobacco cells (3). Very recently (4), this assay also resulted in the identification of two other tobacco peptides, tobacco systemin I and II (Tob sysI and -II). Additionally, their synthesis revealed high similarities to peptide synthesis in animals and yeast.

Our knowledge of peptide signaling in plants was built on the properties of the five peptides discovered so far: tomato systemin, PSK, ENOD40, CLV3, and SCR (1). The latter two are small proteins of 72–75 aa and 53–55 aa, respectively, of which the primary translation products contain an N-terminal hydrophobic stretch that serves as a leader sequence for secretion (5, 6). The ENOD40 primary translation product consists of 10–13 amino acids (7). It is unlikely that CLV3, SCR, or ENOD40 are subject to proteolytic processing to release the biologically active peptide. It has been shown that the active peptides for tomato systemin and *Oryza sativa* PSK are released from a preproprotein by proteolytic processing (8, 9). However, which proteases are involved and their specificity are not known yet. Only the *Os*PSK proprotein has an N-terminal leader sequence (9). With the discovery of RALF and Tob sysI and -II, examples

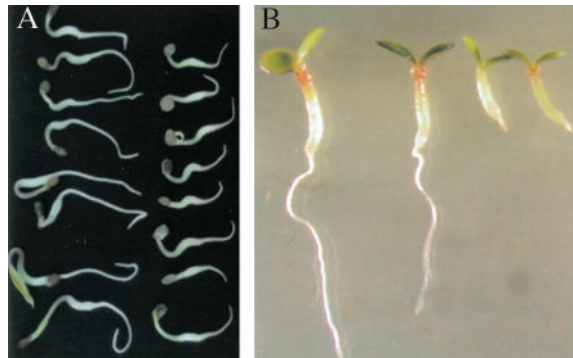


Fig. 1. Tomato seedlings (A) and *Arabidopsis* seedlings (B) exhibit severe arrest of root growth and development when treated with RALF (right seedlings) as compared with wild-type seedlings (left seedlings). Reproduced with permission from ref. 3 (Copyright 2001, *Proceedings of the National Academy of Sciences USA*).

of more stereotypical preproteins in plants have now become available.

Tomato systemin is the first identified plant signaling peptide. It is composed of 18 amino acids and is able to confer a systemic wounding response in tomato plants attacked by herbivores (8). Although more than 100 diverse plant species respond systemically to herbivore attacks (10), homologues of systemin could be identified only in some other *Solanaceae* (11), but strikingly, not in tobacco, which also belongs to the *Solanaceae*. It was known that tomato systemin could activate a mitogen-activated protein (MAP) kinase in suspension culture cells and induce an alkalization of the medium (12). In tomato plants, tomato systemin is able to induce expression of proteinase-inhibitor protein-encoding genes (8). These properties of tomato systemin have been exploited to search for a peptide with systemin-like properties in extracts of herbivore-attacked tobacco leaves using a tobacco suspension culture. By using this assay, two peptides from tobacco were identified that matched all of the criteria for being tobacco systemin analogues and were, therefore, called Tob sysI and Tob sysII (4). Their sequence is not homologous to the tomato systemin, but their function is homologous. From these facts, it can be inferred that structurally diverse peptides in different plant species, although belonging to the same family, can have sim-

ilar functions, an observation not yet made in animals and yeast. The two tobacco systemin peptides are integral parts of a single precursor preproprotein that has an N-terminal leader sequence for translocation via the secretory pathway. The presence of multiple peptides within one precursor molecule is common in animals and yeast, but tobacco systemin is the first example found in plants. During the purification of Tob sysI and -II, RALF was identified as a peptide; like systemin, RALF is able to activate MAP kinase and to induce alkalization of the medium, albeit in a much faster way (3). Therefore, it was named rapid alkalization factor. In contrast to Tob sysI and -II, RALF does not induce proteinase-inhibitor gene expression in plants (3) and, thus, is not a systemin. RALF is part of a preproprotein that contains the typical dibasic motif that, in animals and yeast hormone preproteins, is recognized by specific Ser proteases for the release of the active peptide (13). Therefore, the RALF-containing preproprotein most likely is processed by a Ser protease. In plants, proteins homologous to subtilisin-like proteases, belonging to the super family of Ser proteases, are widespread, but for none of these is

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the cleavage specificity known (13). The RALF preproprotein is, therefore, a good substrate by which to identify the subtilisin-like protease that recognizes dibasic motifs.

RALF homologues have been identified in expressed sequence tag libraries from various organs from 16 species of plants representing nine families; e.g., in *Arabidopsis thaliana*, nine RALF homologues have been identified (3). These findings suggest that the RALF peptides are involved in numerous processes. A first glimpse of the biological functions of these peptides stems from studies in which RALF, applied exogenously, stopped root-growth and root-hair formation (Fig. 1; ref. 3). In these experiments, RALF is active in the nano- to micromolar range, and its effect is reversible, underlining the biological significance of these observations.

The discovery of the new signaling peptides that are part of stereotypical preproteins not only adds more peptides to a growing list of plant peptide-signaling molecules, but also underscores that the principles of peptide signaling in plants and animals do show more similarities than were indicated by the hitherto-studied peptides. The question of whether peptide signaling plays a similar prominent role in plant growth and de-

velopment as in other eukaryotic systems cannot be answered yet. This answer would require a genome-wide search for plant peptides that act as signaling molecules. The discovery of RALF, Tob sysI, and Tob sysII by using a suspension culture system underlines the importance of bioassays in the identification of plant genes encoding peptides. Subsequently, the increasing knowledge concerning plant peptide signaling obtained in this way probably can be used to improve annotation programs.

These observations support the idea that peptide and nonpeptide hormone-activated signaling cascades are linked in plants as they are in animals.

The observed conservation of peptide signaling in plants and animals implies that peptide signaling already was operational in a common ancestor, and that, in time, kingdom-specific features have evolved, such as specific proteases and receptors (14). Concerning the latter, in plants, many genes encoding putative receptors have been identified, whereas in animals, peptide signals are perceived mainly by receptor Tyr kinases and G protein-coupled receptors. Genes encoding such proteins are rare in plants. Instead, plants exploit Ser/Thr kinases coupled to extra-cellular protein domains that, in many cases, contain Leu-rich repeats. Among the latter group can be found receptors involved in plant defense as well as plant developmental processes (15).

For a long time, intercellular communication in plants was thought to be the playground of hormones that have a non-proteinaceous structure. In all known developmental and defense processes, the involvement of one or more of these signaling molecules has been recognized. Although at first glance, these signal molecules seem to be specific to plants; for several of them, structurally related molecules have been identified as signaling molecules in animals. For example, auxin resembles serotonin, ethylene resembles nitric oxide, brassinosteroids are steroids, and jasmonic acid is related to prostaglandins. Further, the involvement of serotonin and insulin in food and metabolic signaling in animals shows that peptide and nonpeptide-activated signaling cascades form networks (16). In plants, it has been shown that the systemin-induced wound response is regulated through the octadecanoid pathway, involving jasmonic acid (17). The PSK-induced cell proliferation requires the hormones auxin or cytokinin (18). Some of the developmental distortions in roots induced on addition of RALF (3) are reminiscent of impaired nonpeptide hormone-controlled processes. These observations support the idea that peptide and nonpeptide hormone-activated signaling cascades are linked in plants as they are in animals. It will be exciting to uncover the links between these two groups of signaling cascades. RALF will be an excellent candidate for this purpose, especially because homologous genes have been identified in the amenable species *Arabidopsis thaliana*.

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