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Archives of Proteomics and Bioinformatics Commentary

Possible Functions of the Conserved Peptides Encoded by the RNA-precursors of miRNAs in Plants

Sergey Y. Morozov[1,2*], Dmitriy Y. Ryazantsev[3], Tatiana N. Erokhina[3]

[1]Department of Virology, Biological Faculty, Lomonosov Moscow State University, Moscow 119234, Russia

[2]Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow 119992, Russia

[3]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Science, Moscow, Russia

[*]Correspondence should be addressed to Dr. Sergey Morozov; morozov@genebee.msu.ru

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Introduction

MicroRNAs (miRNAs) are short double-stranded regulatory molecules derived from precursor transcripts called primary miRNAs (pri-miRNAs) and transcribing in the nucleus from chromosomal DNA by the RNA polymerase II. These pri-miRNAs were shown to contain "cap"-structure and poly(A)-tail and include specific internal imperfect hairpin structures which are processed by DCL (dicer-like) enzyme complexes thus giving rise to miRNA molecules [1-3]. Remarkably, in recent years, pioneer studies of researches from France (UNIVERSITE TOULOUSE III – PAUL SABATIER, Toulouse, and CENTRE NATIONAL de la RECHERCHE SCIENTIFIQUE, Paris) have shown that some plant pri-miRNAs, which previously were considered as non-coding, can be translated and produce small peptides (micropeptides) [4]. Studies on *Arabidopsis thaliana* and *Medicago truncatula* have shown that some pri-miRNAs contain in their 5'-terminal part functional ORFs (open reading frames) encoding the so-called miPEPs (*At*-miPEP165a and *Mt*-miPEP171b, respectively) [4-8]. Importantly, miPEPs were later found to be encoded by the animal pri-miRNAs [9,10].

In general, studies using bioinformatics, GUS (β-glucuronidase) reporter assays, immunoblot techniques, CRISPR (clustered regularly interspaced short palindromic repeats) based editing, as well as ribosome profiling [4,6,10-16] resulted in identification of the novel miPEPs in *A. thaliana* and other plants including *Arachis hypogaea*, *Vitis vinifera* and *Glycine max* [15,17,18]. Our recent works have revealed additional plant micropeptide, miPEP156a, which is evolutionarily conserved in many plants of the family *Brassicaceae* [19,20].

Physiological Effects of miPEPs on Plant Growth and Development

Most studies with miPEPs were performed either with modulating the miPEP ORF expression *in planta*, or with external treatment of plants with synthetic peptides. In general, these works showed that miPEPs may regulate root development, stem growth and flowering parameters [12,14]. Particularly, when *At*-miPEP164a, *At*-miPEP165a and *At*-miPEP319a peptides were applied to plants, the flowering day was decreased with concomitant increase in the length of inflorescence stem [4,7,8]. Root modifications (namely, stimulation of main root growth and decreased lateral root formation) have been found after external application of *At*-miPEP165a and *Mt*-miPEP171b resulting in highly increased cell proliferation in meristematic zone as well as cell elongation [4,7,8,21]. On the other hand, it is known that plant watering with *Gm*-miPEP172c may affect nodulation and cause the increase in nodule number in legumes [18]. Similarly, *Vvi*-miPEP171d1 promotes adventitious root development and restricts primary root development in grapevine when it is applied exogenously or overexpressed [17]. Knock-out mutants of miPEP858a in *Arabidopsis* performed with CRISPR editing technologies showed evident decrease in root length. Whereas, the exogenous treatment of mutant plants with this particular miPEP results in increase of the root length [16]. These experiments also showed that *At*-miPEP858a controls flavonoid biosynthesis and plant development by regulating the expression of genes involved in the phenylpropanoid pathway and auxin signaling [16].

Our experiments with miPEP156a applied to *Brassica* species also showed significant positive effect of miPEP on the primary root growth in seedlings [20].

Potential Molecular Mechanisms of miPEPs Affecting Plant Development

Exogenous application and overexpression of micropeptides resulted in enhanced accumulation of their respective pri-miRNAs, pre-miRNAs and mature miRNAs [6,12,14]. Particularly, micropeptides *At*-miPEP165a, miPEP160b, miPEP164a, miPEP319, miPEP169d, miPEP171e and *Mt*-miPEP171b positively regulated the accumulation of their mature miRNAs [7,8]. In grape, an exogenous application of vvi-miPEP171d1 can enhancing the expression of vvi-MIR171d [17]. Experiments with transcriptional inhibitors strongly suggest that the enhancement of mature miRNA occurs at transcriptional level [4].

Further, promoter activity analysis directly indicated the role of miPEP858a in regulation of its own promoter activity in reporter GUS gene transcription assay [16]. Our experiments with miPEP156a in *Brassica* species also showed evident positive effect of miPEP on the pri-miR156a formation [20]. However, it cannot be excluded that some miPEPs may act not only directly by enhancing own pri-miRNA promoter activity, but also indirectly by regulating transcription of specific transcriptional factors required for the promoter activity.

Tissue-specific Distribution of miPEPs in Plants

Many plant-specific small peptides are considered as long-distance signaling molecules involved in root development [22,23], so it is interesting whether miPEPs can be involved in root-to-shoot communication. Studies of the ability of fluorescently labelled miPEP165a to move in Arabidopsis roots after exogenous application showed that the labelled peptide entered into the epidermis and the pericycle but did not enter the root phloem and move to upper parts of the plant [4]. These data were confirmed in a later study [21] which also suggested that miPEPs cannot migrate throughout the plant. However, in our work with exogeneous miPEP156a [20], it was shown that micropeptide can move actively to the leaves of seedlings. Further studies are required to understand the cause of the above-mentioned contradictions between *At*-miPEP165a and Brassica miPEP156a: either plants specific differences or different modes of action between micropeptides.

Conclusion

Currently, some new questions are being raised in relation to the fine molecular mechanisms underlying miPEPs functions. Particularly, how are the promoter regions interacting with miPEPs to activate pri-miRNA transcription? If this recognition is really taking place, how the transportation of miPEPs across cell wall, plasma membrane, and to the nucleus occurs? Moreover, new studies should be performed to reveal specific aspects of miPEP dynamics concerning changes in subcellular and tissue-specific accumulation of peptides in relation to plant ontogenetic stages. Importance of the additional studies in these directions is highlighted by alternative findings concerning a) subcellular localization of plant miPEPs and b) positive regulation of pri-miRNA/miRNA expression by miPEPs. Particularly, recent studies with human miPEPs showed that induction of pri-miRNA/miRNA expression is not a general rule of microprotein functioning [10]. Nevertheless, due to the quite difference between mammal cell and plant cell, this statement from the human might not be the same to plant cells. In the case of plants, it was shown that *At*-miPEP165a does not move into plant cell nuclei [21] that seems inconsistent with the ability of miPEP165a to activate transcription of own pri-miRNA [4]. Nevertheless, in some other papers, it was found that plant-expressed *Mt*-miPEP171b is localized in small nuclear bodies [8], and exogenous miPEP156a in *Brassica* sp. migrates efficiently into nuclei of phloem and leaf cells [20]. So, it is obvious that a broad comparative investigation of subcellular localization and promoter binding activity of different miPEPs in the diverse plants should be carried in the nearest future.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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