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miRNA-encoded peptides (miPEPs): A new tool to analyze the roles of miRNAs in plant biology

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MicroRNAs (miRNAs) are short RNA molecules negatively regulating the expression of many important genes in plants and animals. We have recently shown that plant primary transcripts of miRNAs encode peptides (miPEPs) able to increase specifically the transcription of their associated miRNA.[1] We discuss here the possibility of using miPEPs as a new tool for functional analysis of single members of miRNA families in plants, including in non-model plants, that could avoid transgenic transformation and minimize artifactual interpretation. We also raise several fundamental and crucial questions that need to be address for a deeper understanding of the cellular and molecular mechanisms underlining the regulatory activity of miPEPs.

Keywords: microRNAs, miRNAs, microRNA-encoded peptides, miPEPs, plant biology

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Introduction

The complex molecular mechanisms that finely tune the general process of protein production through transcription of DNA and translation of RNA are far from being fully understood. Correct implementation of these mechanisms in living organisms is essential for harmonious growth, development and reproduction, and for good adaptability and fitness. As a result, research is extremely active on DNA methylation, chromatin organization, alternative gene splicing, upstream ORFs, lncRNAs or microRNAs (miRNAs).[2-6] The conserved regulation of gene expression involving miRNAs for example, that occurs in plants and animals, is a vital regulation without which mutants unable to generate miRNAs show highly pleiotropic phenotypes or are lethal.[7-10] Silencing of target genes by miRNAs is a highly dynamic process;[6] it includes several steps of miRNA maturation followed by mRNA site-specific cleavage or inhibition of translation. The complexity and precision of this process that is made possible thanks to several conserved proteic effectors suggests that it is indeed the result of a long evolution.[11]

The regulation of transcription of miRNAs is little studied and the role of the primary transcript of miRNA in miRNA biogenesis is poorly understood. The presence of introns has been shown as modulating the maturation of miRNAs, without a full understanding of the exact underlined molecular mechanism.[12,13] We have shown recently the presence of coding ORFs in plant miRNA loci.[1] The synthesized peptides called miPEPs (microRNA-encoded peptides) stimulate the transcription of their associated miRNA, leading to the production of higher amount of miRNA and more pronounced silencing of corresponding target genes. Moreover, the results suggest that this positive regulatory activity of miPEPs is highly specific to their associated miRNA. Interestingly, treatments of plants with synthetic miPEPs can have strong phenotypic effect as a result of a positive regulation of the synthesis of their corresponding miRNA.

Fundamental and Applied Interest of Mipep Technology

The discovery of miPEPs in plants and the possibility to use them in exogenous treatments brings a new and powerful tool to investigate the role of miRNAs. So far, the commonly used strategies for

functional analyses of miRNAs were to study the phenotypes of transgenic plants either by down-regulating the miRNA effect with a target mimicry construction,[14] or by over-expressing the miRNA with a strong promoter like the Cauliflower Mosaic Virus (CaMV) 35S. The target mimicry approach is interesting because only few mutants of miRNAs have been described so far in plants.[15,16] The disadvantage of this approach is that it cannot decrease the activity of only one member of a miRNA family. The overexpression of miRNAs, which is by far the most used approach for probing miRNA functions has the great disadvantage to be ectopic: miRNAs are expressed constitutively all the time and in all tissues, leading to aberrant phenotypes. For example, miR319a is not naturally expressed in leaves but in flowers, and miR319a null mutants are strongly impaired in flower development.[16] In the gain of function *jaw* mutant (for jagged and wavy leaf-phenotype), the ectopic overexpression of miR319a results in the formation of highly serrated leaves, suggesting that miR319a is involved in leaf formation.[17] By contrast, when compared to ectopic miRNA overexpression, treatment with synthetic miPEPs or transgenic overexpression of miPEPs will probably result in weaker phenotypes but these approaches will have strong advantages. Above all, as miPEPs are expected to be active only in cells that are expressing their encoding miRNA. MiPEP treatment or miPEP overexpression should result in more contextual and less artificial phenotype. Moreover, as it has been shown in Extended Table 2 of Lauressergues et al.[1], miPEPs of different members of a miRNA family are all different. Therefore, treatment with synthetic miPEPs or transgenic overexpression of miPEPs will allow distinct functional analysis of each member of miRNA families (Couzigou and Combier, unpublished).

A more applied interest of miPEP technology is that it can be relatively easy to implement on agronomical plants. Simple in silico search and molecular analyses can be sufficient to identify a specific miPEP in a non-model plant. Then, with no need of genetic transformation, just with miPEP exogenous treatment, certain genes targeted by the corresponding miRNA can be down-regulated. By this way, many physiological or developmental phenotypes of agronomical interest could theoretically be modified in crop plants (André and Combier, unpublished).

Next Questions and Perspectives

The identification of miPEPs raises many intriguing questions concerning their prevalence and biology.

One of the main questions is about the universality of miPEPs: do all miRNAs have miPEPs? Particularly are miPEPs present in humans? If so, given that many diseases in humans are caused by insufficient miRNA expression, miPEP technology would be of great interest for the development of new therapeutic strategies. To address the above questions, *in silico* genome investigations and high throughput mass spectrometry technologies to detect small peptides must be developed.

Other crucial questions concern the exact mechanisms by which miPEPs activate their encoding primary transcript: how are they interfering with the transcription machinery? What are the molecular bases of miPEP specificity? What sequence of the primary transcript of miRNAs or of the promoter regions are recognized by miPEPs? As treatments by miPEPs or overexpression of miPEPs never result in a very strong increase of miRNA abundance, it is possible that a negative feed-back mechanism, yet to be described, controls the effect of miPEPs. Primary transcripts are capped and polyadenylated RNA molecules that are rapidly recruited by the dicing complex. How can they be translated to produce miPEPs? Whereas some nuclear translation has already been reported,[18] the presence of at least some miRNAs in ribosome profiling data suggests the occurrence of other mechanisms,[19] but it is unclear how primary transcripts of miRNAs can be found in the cytoplasm.

Contrary to mature miRNAs, which are very well conserved in the plant kingdom, miPEPs appear to be generally quite variable across plant genera (data not shown). The only exception found so far is for miPEP165a which seems to be fairly well conserved among Brassicales.[1] This intriguing observation will probably find some explanation when the mechanisms of miPEP activities will be fully understood and when, from this knowledge, the type of selective pressure undergone by miPEP-encoding ORFs may be deduced. From a more applied viewpoint, this feature is extremely interesting. If a given miPEP is developed for a certain agronomic activity on one particular crop it should be specific.

Finally one intriguing question is how miPEPs can penetrate in plants, at least in roots, cross the secondary cell wall, the plasma membrane, and then enter the nucleus. Do cells have some miPEP transporters allowing their penetration into cells? Moreover, due to their apparent high mobility, we could ask whether a long distance mobility of miPEPs in plants exists and would allow a communication between their different parts.

In the last years, a growing number of studies have shown that genomic sequences previously thought to be non-coding in fact encode small peptides.[20-28] By using ribosome profiling.[20-21] or peptidomic.[22] analyses, the authors highlighted that long non-coding RNAs or small Open Reading Frames can produce peptides, some of them having important regulatory functions.[23-28] MiPEPs are an additional example of peptides with strong biological function whose encoding sequences were well hidden in the genome. To see the forest rather than the trees, development of easy and reliable mass spectrometry methods allowing the identification of entire peptidomes is urgently required.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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