

Microproteins – A recent resource for the regulation of protein functions in crop plants

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Abstract

A microprotein (miP) is a small protein encoded using the small open reading frame (smORF). Microproteins regulate larger multidomain proteins at the posttranslational stage. Microproteins, like microRNAs (miRNAs), heterodimerize with their targets, causing dominant and antagonistic effects. Microproteins have been identified to affect biological processes in both animals and plants. Because of their dominant effects on their targets, microproteins are currently being studied for future applications in biotechnology.

Keywords: microprotein, miP, protein function, regulation

Introduction

Micro Proteins are tiny proteins that contain no additional functional domains and having either a single protein domain or protein-protein interaction (PPI) domain. By creating functional heterodimers or including the target protein in new protein complexes, microproteins can either totally inactivate their targets or change their biological function. The first microProtein is the DNA BINDING INHIBITOR (Id) was discovered in mammals. It is a 16kDa tiny, helix-loop-helix (HLH) domain protein. The first microproteins described in plants were LITTLE ZIPPER (ZPR) proteins. ZPR proteins have a domain with a leucine zipper but lack any additional domain needed to bind and activate DNA. ZPR proteins are therefore used to govern developmental processes such as stem cells maintenance in the shooting of apical meristem (SAM) and leaf development by analogy with the proteins of an

Id-type and interact physically with class III homeodomain-leucine zip (HD-ZIP III) (Bhati et al., 2018).

Concept, classification and functions of Microproteins: Based on the original method, microproteins are classified into two kinds. The first is Trans-Micro Proteins, the single transcription unit evolutive associated with more multiple genes encode multidomain proteins. Some micro-protein genes have been developed through genome amplification and subsequent domain loss events and have produced tiny inhibitory proteins having a single domain. The second is that the mRNA isoforms encode micro proteins, which arise from mechanisms like splicing, alternate translation start and stop site selections. Furthermore, post-translation modifications may also create microProteins, such as proteolytic cleavage, leading to smaller molecules capable of interfering with their more significant precursors. Microproteins sometimes act as negative regulators. For instance, LITTLE ZIPPERS have HD-ZIIP III transcription control but negatively regulate non-productive dimer proteins of the HD-ZIP III transcription factor (Bhati et al., 2018).

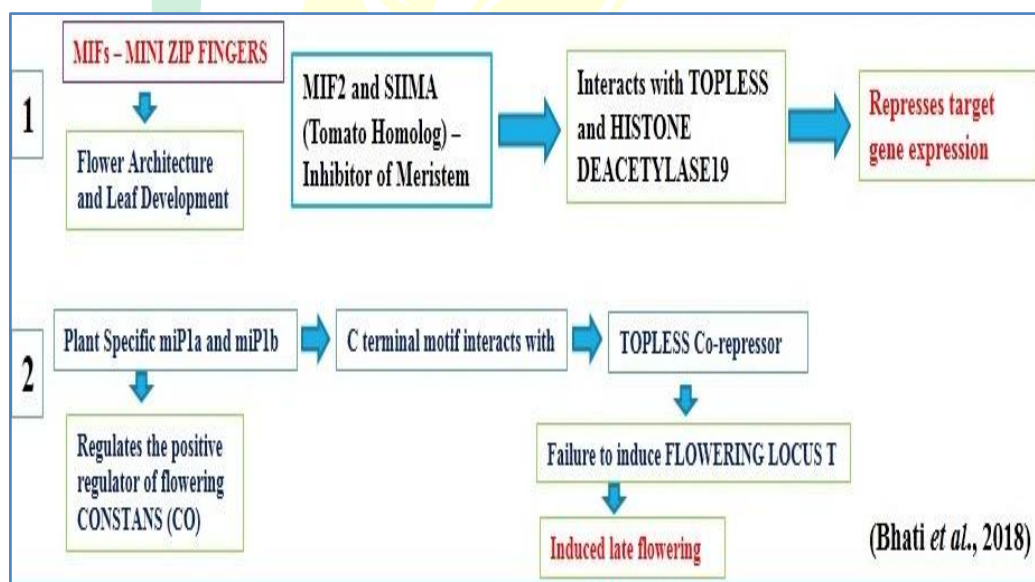


Figure 1. Some examples of the mode of microprotein regulation

The plant Id-like KDR protein sequester HFR1 shifts balance to PIFs and enables active homodimer to be formed. Overexpression of the target bHLH (IBH1) produces a lower leaf-lamina inclination in rice and dwarfism. In contrast, over-expression of the Id-like protein PRE or ILI1 raises the leaf-lamina inclination in rice (Staudt and Wenkel, 2018). Leucine Zipper like micro proteins are also available in plants. HD ZIP III transcription factors in wild plants bind to DNA as homodimers to govern development processes such as meristems and the formation of leaves. The meristem gets more disordered and can be altered in hd-zip III gain-of-function mutant plants or multiple ZPR loss-of-function mutant plants. In case of reducing HD ZIP III activity or loss of ZPR protein, the photo-meristem may end, and leaves can be shown curling downwards (Staudt and Wenkel, 2018).

Targets for miPs in plant species are homeodomain transcription factors belonging to the Tri-amino-acid loop-extension family (TALE). Transcription regulator TALE Homeodomain is defined by insertion in the homeodomain of three amino acids α -helices 1-2. Homeodomain TALE proteins are developed in mammals, fungi, and plants. They are conserved to indicate that they arose in a common ancestor. In plant KNOX genes, the KNOTTED domain (KNOX) is preserved. Two KNOX genes are present in Arabidopsis, each including four genes encoding MEINOX-TALE-homeodomain proteins known to be KNOX proteins. Plant KNOX proteins physically bind with BEL-like homeodomain proteins (BELLs), whereas mutant studies show this association is working (Bhatt et al., 2004; Smith & Hake, 2003).

Mode of microprotein regulation : When MIF's are evaluated, the architecture and the growth of flowering plants are closely connected to them, but MIF2 and SIIMA (Fig 1) interact with TOPLESS and HISTONE DEACETYLASE19 repression the expression of target genes. In this way, plant-specific miP1a and miP1b favourably control the flora of agricultural plants (Fig 1). At the same time, the C-terminal motif interacts with the TOPLESS corepressor and promote late-blooming (Bhati et al., 2018). There are several ways of regulating microproteins. The first is to sequence their targets into non-functional complexes (e.g. Id/Id as ZPR). The second is by repressing the protein of the repressor (for example – MIF2 or miP1a/b). The second is through repression. The third one, by sequestering the target on a subcellular chamber where it is inactive (example – MIF), is

cytoplasmic retention. The fourth is to engage with and compromise the transport capacity of ion channels by interaction with ion channel subunits (Example – VPU).

Working with a prominent approach for identification of microproteins (miPFinder): A unique method in bioinformatics miPFinder is the discovery of four ways of microproteins (Fig 2). The biotechnological importance of microproteins to regulate protein activity is specific. Arabidopsis resulted in a phenotype comparable to that of the loosen-function mutant of the respective transcription factors in the overexpression of a PPI domain for SUPPRESSOR of OVEREXPRESsOR of CONSTANS1 (SOC1), AGAMOUS(AG), and LATE ELONGATED HYPOCOTYL (LHY) etc. Synthetic microproteins were also utilised to regulate the blooming period of long-day rice (Seo et al., 2012; Eguen et al., 2018).

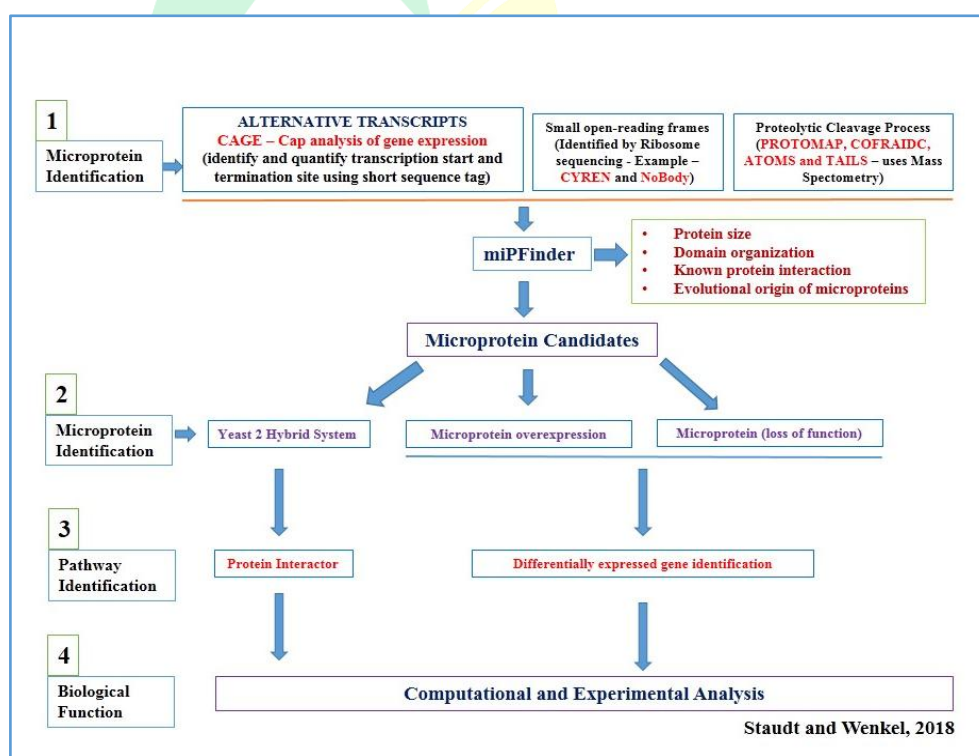


Figure 2. Working with miPFinder - a prominent approach for identification of microproteins

Conclusion: Small proteins with a single protein domain linked to more prominent, typically multidomain proteins are microproteins. Microproteins work at the molecular level by interfering with the forming of higher-order complex proteins. Several microproteins in plants and animals that impact biological processes have been discovered in the last years. Because they can serve as a dominant regulators in a tailored way, biotechnological usage of microproteins has great promise.

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