Target mimics modulate miRNAs

Daniel H Chitwood & Marja C P Timmermans

A 23-nucleotide sequence conserved in species from rice to *Arabidopsis thaliana* in a family of noncoding RNAs resembles a cleavable miRNA target site—but not exactly. A new study demonstrates that the site is not cleaved and instead negatively regulates miRNA activity through mimicry.

MicroRNAs (miRNAs) are small RNA species that regulate gene expression. The hairpin structures of noncoding miRNA primary transcripts are processed to yield mature miRNA molecules that regulate target gene expression through transcript cleavage and translational repression. Recently, attention has been focused on how biogenesis mechanisms and additional transcriptional regulation restrict the accumulation of miRNAs, spatially and temporally, to direct developmental and physiological responses. However, hypotheses of how the activity of the final effector miRNA molecule can be regulated are lacking. That such potent regulators of gene expression would remain unbridled seems unlikely, given the numerous restraints placed upon their protein analogs. On page 1033 of this issue, José Manuel Franco-Zorrilla *et al.*[1] demonstrate that a noncoding transcript in *Arabidopsis thaliana* regulates the activity of an miRNA implicated in phosphate homeostasis by mimicking its target site. Beyond suggesting an interesting means by which miRNAs are naturally regulated, the work shows that target mimics can artificially regulate miRNAs, introducing a powerful tool to study miRNAs genetically in plants and, perhaps, in animals.

Starving for P_i

Plants respond to inorganic phosphate (P_i) deprivation through a variety of means, including remobilization of P_i between the shoot and root and changes in root architecture[2,3]. PHOSPHATE2 (PHO2), an E2 ubiquitin-conjugating enzyme, is an important negative regulator of these responses that is downregulated during P_i starvation[4–7]. The mechanism of *PHO2* repression is critical to understanding the induction of the P_i starvation response and centers on an miRNA, miR399. Steady-state miR399 levels increase robustly during P_i starvation, during which this miRNA cleaves *PHO2* transcripts. The importance of miR399–mediated *PHO2* cleavage is exemplified by the numerous (up to five) miR399 target sites in the 5′ UTR of *PHO2* that are conserved throughout the angiosperms[1,7].

The downregulation of *PHO2* by miR399 during P_i starvation must be transient—quick to respond to starvation but also quick to recuperate to normal levels afterwards, to prevent P_i toxicity. This requires that an attenuator of miR399 activity be present during the starvation response, such that after P_i-replete conditions return, miR399 activity is rapidly cleared. Additionally, although *PHO2* activity decreases during P_i deprivation, the absence of *PHO2* is not necessarily desirable; in fact, it does not occur. Time-course experiments show an increase in *MIR399* precursor levels and a concomitant decrease in *PHO2* transcript levels upon P_i deprivation[7] (**Fig. 1**). However, after this initial decrease, *PHO2* transcript levels actually stabilize, despite the fact that *MIR399* precursor levels continue to rise. The results are consistent with the delayed activation of an miR399 inhibitor that attenuates miR399 activity and tempers the reduction of *PHO2* transcript levels.

One RNA negatively regulates another

Until work by Franco-Zorrilla and colleagues[1], the identity of the hypothetical attenuator and the mechanism by which it acted remained unknown. Surprisingly, one candidate is a noncoding P_i-responsive transcript first discovered in tomato[8] (*TPSI*) and *Medicago*[9] (*Mt4*). The only homology between *Mt4-TPSI* family members is a 23-nucleotide motif that is almost, but not quite, complementary to miR399[10]. Franco-Zorrilla *et al.* observed in all *Mt4-TPSI* family members a 2- to 4-bp mismatch in the miRNA target site, disrupting its complementarity between bases 10 and 11 of the miRNA—exactly where cleavage is expected to occur. Functional noncleavable miRNA target sites are not unknown: the *TAS3* precursor loci

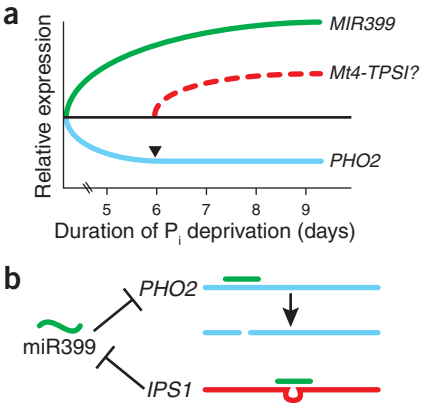

Figure 1 Possible models of how the induction of the P_i deprivation response in plants is regulated by an miRNA and its target mimic. (**a**) Upon P_i deprivation, *PHO2* transcript levels decrease as *MIR399* precursor levels increase. However, *PHO2* levels eventually stabilize (arrowhead), suggesting the existence of a hypothetical inhibitor of miR399 activity. One possible candidate for the inhibitor is the *Mt4-TPSI* family of transcripts, the delayed expression of which might account for the metastable state of *PHO2*. (**b**) Mechanism of target mimicry. miR399 recognizes a noncleavable target site on *IPS1*, effectively sequestering and inhibiting the cell's population of miR399 molecules from cleaving *PHO2* target transcripts. Panel **a** is adapted from ref. 7.

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that encode *trans*-acting siRNAs (ta-siRNAs) possess two miR390 target sites, one of which is not cleaved but rather is required for ta-siRNA production[11].

To test the significance of the noncleavable miR399 target site in the *A. thaliana Mt4-TPSI* family member *IPS1*, the authors began by constitutively expressing miR399. As expected, *PHO2* transcript levels decrease and shoot P_i content increases, similar to the phenotype of *pho2* mutants[4–6]. However, when they simultaneously overexpressed *IPS1*, they saw that these effects were partially counteracted, consistent with *IPS1* inhibiting the activity of miR399. Importantly, an *IPS1* transgene mutated to be perfectly complementary to miR399 is not only cleaved but also lacks any inhibitory effects. This result is consistent with the long-standing observation that overexpression of miRNA target genes rarely yields notable results, supposedly because the target is simply cleaved and cleared.

To explore the mechanism by which *IPS1* acts as an inhibitor of miR399 activity, the authors created a heterologous system in tobacco leaves. When a *PHO2-GFP* reporter and miR399 are transiently coexpressed, *PHO2-GFP* does not accumulate as a result of miR399-mediated *PHO2* cleavage. When *IPS1* is also coexpressed, GFP fluorescence is restored, reflective of the role of *IPS1* as an inhibitor of miR399 activity. Mutations in the miR399 complementary site of *IPS1* abrogate this ability, allowing miR399 to again cleave *PHO2-GFP*. If compensatory mutations are made so that an artificial miRNA recognizes a mutated *PHO2* target site as well as the mutated noncleavable site in *IPS1*, the system acts exactly as Franco-Zorrilla *et al.*[1] propose it should act *in vivo*: an miRNA target mimic effectively competes for miRNA activity (**Fig. 1**).

A genetic tool

That an artificial noncleavable miRNA target site is capable of inhibiting the activity of a complementary artificial miRNA suggests a means of knocking down miRNA activity *in vivo*. Franco-Zorrilla *et al.*[1] engineer *IPS1* to do just that, knocking down miR156 and miR319 activity by overexpressing mutated *IPS1* transgenes with noncleavable target sites for these miRNAs. In these plants, targets of miR156 or miR319 show increased accumulation, as would be expected if miRNA activity were reduced.

This proof-of-principle experiment has major implications for the *in vivo* study of miRNAs. Loss-of-function mutations for single miRNA family members have little phenotypic effect[12]. This is because miRNA families—especially in plants—are large, with many loci encoding identical, or negligibly different, miRNAs. The overexpression of a noncleavable target circumvents the tedious task of constructing multiple mutant backgrounds and allows for loss-of-function experiments to study the natural contributions of miRNAs to biological processes by blocking their activity.

Riboregulation

How prevalent is this mechanism of target mimicry? That miRNAs can efficiently clear a cell of target transcripts makes them attractive effectors of rapid physiological and developmental changes. However, tempering such potent regulators of gene expression allows more precise control over biological processes. Are other miRNAs that regulate physiological responses (such as miR395 in sulfate metabolism and miR398 in oxidative stress) similarly regulated by target mimics[13]? Target mimics could also feasibly act during development, modulating miRNA activity to tune targets to defined, stable levels of expression, providing an alternative scenerio of genetic regulation to the widespread clearance of targets by developmental miRNAs. The answer to the question of whether target mimicry is prevalent or unique to miR399 will start with the computational identification of other putative target mimics, especially by relaxing search parameters to allow mismatched bulges near the target cleavage site.

The idea of target mimicry demonstrates unanticipated complexity in the network of RNA regulatory interactions. Not only has a tangible function been ascribed to another long, noncoding RNA, but it is an example of self-regulation by RNAs, a property of the proteome taken for granted. The modulation of the extent of an inductive response through the coexpression of an attenuator is not a new concept; it commonly confers tractability to protein signaling networks[1]. It will be interesting to see how many of the myriad noncoding RNAs function as riboregulators through interactions with other small RNAs.

COMPETING INTERESTS STATEMENT
The authors declare no competing financial interests.

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Axons need glial peroxisomes

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Several devastating genetic diseases illustrate that peroxisomes are essential to the development and functioning of the central nervous system. New work using a mouse model now shows that peroxisome integrity in oligodendrocytes is essential for axonal maintenance.

First described by Baudhuin and de Duve in the mid-1950s, peroxisomes are single, membrane-bound, typically spherical organelles ranging in size from 0.1 μm to 1 μm in diameter and ranging in number from a few hundred to a few thousand per mammalian cell. The dense, amorphous peroxisome matrix contains more than 50 enzymes that participate in the β-oxidation of 2-methyl branched-chain and very-long-chain fatty acids, the synthesis of ether lipids (plasmalogens) and the oxidation of D-amino acids and polyamines. H_2O_2 produced by peroxisomal oxidases is detoxified *in situ* by peroxisomal catalase. The machinery that imports peroxisomal enzymes into the peroxisomal matrix is highly flexible and includes the cycling receptor peroxin 5, encoded by *Pex5*. Genetic defects of *PEX* genes in humans result

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