P205

Use of Transiently Expressed RNAi in Dissecting Hormone Signaling Pathways in Cereal Grains

Tuan-hua David Ho

Department of Biology, Washington University, St. Louis, Missouri, USA

Double-stranded RNAs (dsRNAs) can be used to silence the expression of target genes in a variety of organisms and cell types (e.g., worms, fruit flies, and plants). We have adopted this technique in transient expression manner to study hormone signaling pathways in the aleuronecells of cereal grains. Specific RNAi molecules are generated in these cells via the biolistic introduction of transgenes containing inverted repeats of the target sequences. It has been shown that the gene silencing effect reaches a maximum within 4 hrs of introducing the RNAi generating transgene, and one copy of the RNAi-generating transgene is capable of knocking down the expression of more than 10 copies of the target gene. In the cereal aleurone tissue, the phytohormone gibberellin (GA) induces and another hormone abscisic acid (ABA) suppresses the expression of α-amylases that are essential for the utilization of starch stored in the endosperm. Following the RNAi-mediated gene silencing approach, it is demonstrated that the transcription factor, GAMyb, is not only sufficient, but also necessary for the GA-induction of α-amylase. Another regulatory protein, SLN1, is shown to be a repressor of GA action, and knocking down the synthesis of SLN1 by RNAi leads to derepression of α-amylase even in the absence of GA. However, this effect is still suppressed by ABA. Although the ABA-induced serine/threonine protein kinase, PKABA1, is known to suppress the GA-induced α-amylase expression, *PKABA1*RNAi does not hamper the inhibitory effect of ABA on the expression of α-amylase, indicating that a PKABA1-independent signaling pathway may also exist. We have also demonstrated that ABA induction and suppression of gene expression follow two distinct signaling pathways. Based on these promising results, we suggest that the generation of specific RNAi in a transient expression approach is a useful technique in elucidating the role of regulatory molecules in biological systems in which conventional mutational studies cannot be easily carried out.