Seminars in Cell & Developmental Biology 56 (2016) 190–200

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Small RNAs regulate plant responses to filamentous pathogens

Tung Kuan [a,b,1], Yi Zhai [a,b,1], Wenbo Ma [a,b,*]

[a] Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521, USA
[b] Center for Plant Cell Biology, University of California, Riverside, CA 92521, USA

ARTICLE INFO

Article history:
Received 11 March 2016
Received in revised form 10 May 2016
Accepted 17 May 2016
Available online 18 May 2016

Keywords:
Plant filamentous pathogens
Small non-coding RNA
RNA silencing
RNA silencing suppressors

ABSTRACT

Small RNAs are central players of RNA silencing in eukaryotes. These short RNA molecules (20–25 nucleotides in length) repress target gene expression based on sequence complementarity. While small RNAs are well-known for their essential function in regulating growth and development, recent research has revealed that they also influence plant immunity. Extensive changes in small RNA accumulation have been observed during infection. This review focuses on specific small RNA changes that are involved in plant responses to filamentous eukaryotic pathogens including fungi and oomycetes. We describe how changes in small RNA accumulation influence plant immunity and summarize the cellular processes affected by these small RNAs. In particular, we discuss secondary small interfering RNAs that directly modulate the expression of defense-related genes.

Contents

* Corresponding author at: Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521, USA.
E-mail address: wenbo.ma@ucr.edu (W. Ma).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.semcdb.2016.05.013
1084-9521/

1. Introduction

Constantly challenged by potential microbial pathogens in the surrounding environment, plants have evolved two branches of immunity to prevent infection [1,2]. The first branch relies on the recognition of microbial- or pathogen-associated molecular patterns (MAMPS or PAMPs) by transmembrane proteins called the pattern recognition receptors (PRRs) [3]. Activation of PRRs leads to defense responses, including reactive oxygen species (ROS) production, cell wall reinforcement (callose deposition) and antimicrobial compound secretion. This pattern-triggered immunity (PTI) serves as a general or basal defense that prevents the colonization of the majority of potential pathogens; however, successful pathogens are able to effectively defeat PTI through the function of effectors. It became clear that effectors are produced by a broad range of plant pathogens and their major function is to suppress host immunity [4]. As a counteractive strategy, plants evolved another layer of defense, which depends on the recognition of specific pathogen effectors by resistance (R) proteins in a gene-for-gene manner [5]. Canonical R proteins share conserved nucleotide-binding leucine-rich repeat (NB-LRR) domains, and the activation of the NB-LRR proteins results in effector-triggered immunity (ETI). ETI often involves programmed cell death called the hypersensitive response (HR), which restricts the spread of the pathogen from initial infection sites [2].

Both PTI and ETI involve defense signal transduction through kinases (such as mitogen-activated protein kinases or MAP kinases) and extensive transcriptional reprogramming, which eventually leads to immunity [6]. This process requires precise regulation due to its high energy consumption that inevitably affects plant growth [7,8]. There is an accumulating body of evidence suggesting that small non-coding RNAs are integral regulators of defense-related gene expression during pathogen infection as well as a pivotal switch that governs the growth/defense tradeoff [9–11].

Small RNA silencing is a universal and fundamental gene regulation mechanism in eukaryotes that governs cellular processes. In plant immunity, it is well-established that virus-induced RNA silencing is critical for anti-viral defense [12,13]. More recent studies showed that specific small RNAs were differentially accumulated during infection by bacteria, fungal and oomycete pathogens [10,14]. Small RNAs have also been found to suppress PTI and ETI in the absence of pathogens to avoid autoimmune responses [15,16]. Furthermore, effectors with small RNA silencing suppression activity have been identified from bacteria [17] and oomycetes [18,19]. These findings strongly suggest that small RNA silencing is required to establish effective defense response to a large variety of pathogens.

Plant pathogens are generally divided into biotrophs and necrotrophs based on their infection styles. Biotrophic pathogens establish a complex symbiosis relationship with specific hosts and feed on living tissues; on the contrary, necrotrophic pathogens kill plant cells and obtain nutrients from dead/collapsed tissues of a broad range of hosts [20]. Responding to these different infection strategies, plants utilize distinctive defense mechanisms [20,21]. In this review, we will focus on biotrophic and hemi-biotrophic (containing an early biotrophic infection stage and a late necrotrophic infection stage) pathogens due to their complex molecular interactions with the hosts. Furthermore, we will focus on the filamentous eukaryotic pathogens as there is emerging body of evidence that suggest a particularly important role of small RNAs during plant defense against these destructive pathogens, including fungi and oomycetes.

2. Evidence suggesting small RNA silencing influences plant immunity

2.1. Plant mutants defective in small RNA silencing exhibited altered susceptibility

Plants produce two major classes of endogenous small RNAs, namely microRNAs (miRNAs) and small interfering RNAs (siRNAs) [11]. miRNAs mediate sequence-dependent post-transcriptional gene silencing (PTGS) by guiding mRNA cleavage and/or translation inhibition; whereas siRNAs can also mediate transcriptional gene silencing (TGS) through sequence-dependent DNA modification in addition to PTGS [9,22]. The core components of plant small RNA silencing pathways include Dicer-like ribonucleases (DCLs) that produce small RNAs from double stranded precursors, Argonaute (AGO) proteins that form the RNA silencing effector complexes, RNA-dependent RNA polymerases (RDRs) that synthesize the double stranded precursors, and double-stranded RNA binding proteins (DRBs) that facilitate small RNA biogenesis [11]. Genes encoding these core components have been characterized for their effect on plant defense during pathogen infection.

2.1.1. miRNA pathway

miRNAs, mainly 21 nucleotides in length, are produced from endogenous *MIR* loci, where non-protein coding transcripts are transcribed by RNA polymerase II and form foldback precursors [11,23]. The primary miRNA transcripts (pri-miRNAs) are processed by Dicer-like 1 (DCL1) to generate double-stranded miRNA duplexes in the nucleus. These miRNA duplexes are stabilized by HEN1 methyltransferase and exported into cytoplasm where one strand of the duplex is incorporated into AGOs [11,23]. In plants, most miRNAs are associated with AGO1 (Fig. 1A).

Several plant mutants that are defective in the miRNA pathway showed altered susceptibility upon infection of filamentous pathogens. For example, silencing of *OsDCL1* in rice led to enhanced resistance to the blast fungus *Magnaporthe oryzae* [24]. On the contrary, two *dcl1* mutants in *Arabidopsis thaliana* were hypersusceptible to the oomycete pathogen *Phytophthora capsici* [19]. These variable phenotypes could be due to the significant changes in plant morphology caused by *DCL1* mutations, which may exert different effects on individual interactions between specific pathogens and the hosts. In addition to *DCL1*, mutations in *AGO1* and *HEN1* in rice also led to altered resistance to *Verticillium dahliae and Verticillium longisporum* [25,26]. Since AGO1 and HEN1 are involved in both miRNA and siRNA pathways, these phenotypes are more complicated to interprete. Nonetheless, these observations indicate that the miRNA pathway may contribute to the regulation of plant immunity.

2.1.2. siRNA pathway

Distinct from miRNAs, siRNAs are derived from invading nucleic acids such as viruses and transgenes, and endogenous loci such as repeats, transposable elements, and genes [11]. Typically, the precursors of siRNAs are long double-stranded RNAs synthesized by RDRs facilitated by Suppressor of Gene Silencing 3 (SGS3); and three DCLs in *Arabidopsis* catalyze the formation of 21-nucleotide (DCL4), 22-nucleotide (DCL2), and 24-nucleotide (DCL3) siRNAs. The 21-nt and 22-nt siRNAs guide gene silencing by PTGS; whereas the 24-nt siRNAs lead to TGS through the RNA-directed DNA methylation (RdDM) pathway [14,22].

siRNA-mediated PTGS has been implicated in plant immunity during the infection of filamentous pathogens [27]. For example, upon the infection with *M. oryzae*, expression of *OsRDR6* and *OsSGS3* was highly induced in a resistant cultivar of rice while such induction was not observed in a susceptible cultivar [28]. A similar induction of *RDR1* and *RDR6* was also observed in *Nicotiana*