Plant Science 175 (2008) 741–746

Contents lists available at ScienceDirect

Plant Science

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

Review

Processing precursors with RNase III in plants

Gabriela Olmedo, Plinio Guzmán *

Departamento de Ingeniería Genética de Plantas, Cinvestav, Campus Guanajuato, Km 9.6 Libramiento Norte, 36821 Irapuato, Guanajuato, Mexico

ARTICLE INFO

Article history:
Received 27 May 2008
Received in revised form 8 July 2008
Accepted 8 July 2008
Available online 17 July 2008

Keywords:
RNase III
Dicer-like
microRNAs
Arabidopsis thaliana
Mini-RNase III

ABSTRACT

RNase III endonucleases cleave double-stranded RNA to produce different classes of mature RNAs that participate in pre-mRNA splicing, RNA modification, translation, gene silencing, ribosomal biogenesis, and regulation of developmental timing. The approximately 120-amino acid long endonuclease domain (endoND) is the hallmark of this family of proteins, generally recognized by a nine amino acid signature within the domain. The endoND module occurs in a single or double copy in different classes of proteins, alone or with other domains that provide versatility to act in different situations requiring RNA processing. Two groups of proteins containing the endoribonuclease domain can be described in plants. One group represents the RNase III-like enzymes called Dicer-like (DCL) that generate small RNA molecules of specific lengths from longer precursors. There are four classes of DCL enzymes each class producing predominantly one particular processing product. The other group includes non-dicer-like enzymes (non-DCL) with various types of recently described enzymes most of which have not been functionally characterized. Non-DCL comprise the RNase III-like enzymes (RTL1, 2 and 3) identified in *Arabidopsis thaliana*, the RNC1 RNase III enzyme isolated from maize and the *in silico* predicted Mini-RNase III that consists only of an endonuclease domain. RTLs and RNC1 participate in development, splicing, and responses to biotic stress. Mini-RNase III enzymes await functional characterization in plants.

Contents

1. Introduction

RNA molecules often require processing before functioning within the cell. Intron removal in eukaryotes is a common mode of messenger RNA (mRNA) processing that generates a translatable mRNA. Some transfer RNA (tRNA) molecules also require intron removal and elimination of 5′ and 3′ sequences to generate a functional tRNA. A mature ribosomal RNA (rRNA) is synthesized as a precursor that undergoes several steps of processing. Likewise, large double-stranded RNA (dsRNA) molecules are precursors of short interfering RNAs (siRNAs) and microRNAs (miRNAs). Many enzymes involved in RNA processing have been identified. Key components involved in maturation of RNA precursors are ribonuclease III (RNase III)-type of enzymes, which are present in organisms from all kingdoms [1].

* Corresponding author. Tel.: +52 462 62 39662; fax: +52 462 62 39650.
E-mail address: pguzman@ira.cinvestav.mx (P. Guzmán).

0168-9452/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.plantsci.2008.07.001

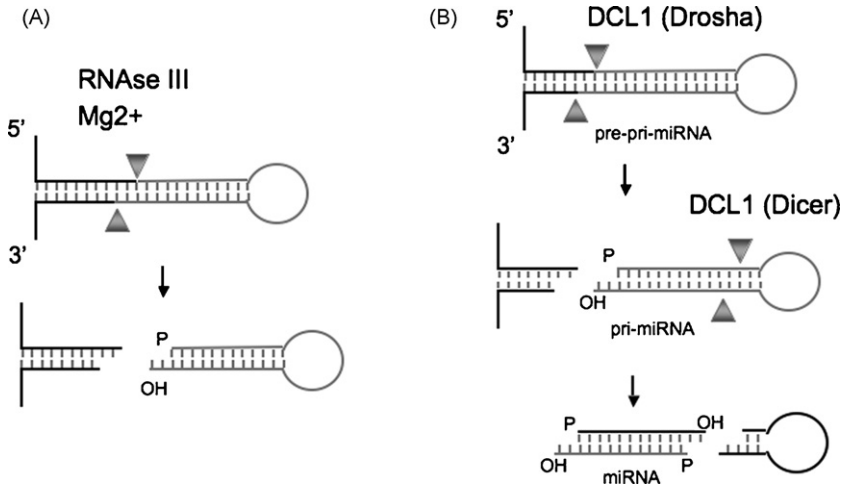

Fig. 1. Schematic representation of the cleavage reaction of RNase III. (A) *E. coli* RNase III cleaves phosphodiester bonds in a Mg^{2+}-dependent reaction [5] leaving a 5′ phosphate and a 3′ hydroxyl end. A 2-nucleotide 3′ overhang is a trademark of the cleavage by RNase III enzymes. A double stranded RNA is the substrate for the reaction, and this is often formed intramolecularly by folding of an RNA strand. The arrows represent the site of cleavage by a dimeric endoND, with each endonuclease domain promoting cleavage of a single strand of the duplex. (B) Two-step process for the generation of miRNAs. Generation of the final product requires at least two cleavages of the substrate, both of which can be carried out by DCL1 in plants [34], but require the participation of Drosha and Dicer in humans. For DCLs (as well as Dicer and Drosha in humans) the two endoNDs form intramolecular dimers, equivalent to those of single endoND domain dimers. 5′ P and 3′ OH are shown only for functional cleavage products.

RNase III enzymes are the most important endoribonucleases specific for dsRNA. They are phosphodiesterases that hydrolyze phosphodiester linkages, generating 5′-phosphate, 3′-hydroxyl products from their substrates. Their substrates are often hairpins with duplex regions and the cleavage generates nicks or staggered breaks (Fig. 1) [2]. RNase III was initially found in *Escherichia coli* extracts in the late 1960's, and its specific role in RNA processing in a number of bacteriophages and bacterial RNAs was then ellucidated [3–5]. RNase III is the best-studied enzyme involved in rRNA processing and is highly conserved in eubacteria, with orthologs in fungi, plants, and animals [1].

More recently, the function of RNase III in processing precursors of short interfering RNAs (siRNAs) and microRNAs (miRNAs) in both animals and plants has boosted the interest in this particular type of ribonucleases. The common feature for all RNase III enzymes is the RNase III catalytic or endonuclease domain (endoND), which is present as either a single or a double module. Generally, RNase III enzymes bind to and process dsRNA into double stranded cleavage products that typically have a two-nucleotide 3′ overhang [6]. A subsequent cleavage reaction at an adjacent site takes place to generate shorter dsRNA molecules such as siRNAs and miRNAs (see Fig. 1). The majority of RNase III enzymes also contain a sequence called the dsRNA-binding domain (dsRBD). This 70-amino acid domain forms a tertiary structure that interacts with dsRNA, generally without obvious RNA sequence specificity [7]. The dsRBD motif is important in dsRNA substrate recognition, but not for the cleavage reaction [8,9]. Finally, although RNase III enzymes usually bind and process dsRNA, there are also examples of RNase III proteins affecting gene expression by binding to, but not cleaving the dsRNA substrate [6,10,11].

The Dicer class of RNase III enzymes has been the focus of several reviews [1,12–14]. Three types of RNase III enzymes structurally more similar to the class 1 RNase III from bacteria and fungi than to Dicer enzymes have been recently described in plants. These newly identified RNase IIIs are predicted to be unique to plants and to have novel functions.

2. Conventional classes of RNase III

Multiple architectures of RNase III containing proteins have been identified. Those consisting of a single endonuclease domain and requiring a partner to dimerize, contrast with those with two endonuclease domains that work as intramolecular dimers. These proteins range in size from the minimal protein consisting of only the endonuclease domain to larger proteins combining modules of diverse function. RNase III enzymes have been grouped in four classes based on the presence (or lack) of additional domains within the protein (Table 1). Class 1 is typically represented by the *E. coli* RNase III and the *Saccharomyces cerevisiae* Rnt1p. Proteins in this class contain a single endonuclease domain at the amino-terminal end and a single dsRBD at the carboxyl-terminal end; the yeast enzyme has an amino-terminal extension that comprises 36% of the entire protein and functions as a modulator of enzyme activity by mediating both inter- and intramolecular interactions [15]. Class 2 is represented by the *Drosophila* Drosha enzyme; it contains two endoNDs, a single dsRBD and a long amino-terminal extension. Class 3 corresponds to the Dicer type of enzymes, which contain the two endoNDs and a single dsRBD, in addition to a helicase domain at the amino-terminal end, and PAZ (*P*iwi *A*rgonaute *Z*wille) and DUF238 (*D*omain of *U*nknown *F*unction

Table 1
Classes of RNase III enzymes

	Organisms	Representative	Endo ND	dsRBD	Other domains	Function
Class 1	Bacteria, some fungi	Ec, RNase III	Single	Single	Absent	Processing precursors of rRNA, tRNA, polycistronic mRNA
Class 2	Animals	Dm, Drosha	Double	Single	Poly-proline	Primary (pri)-miRNA processing
Class 3	Animals, some fungi, plants	Hs, Dicer	Double	Single	Helicase, DUF283, PAZ	miRNA biogenesis
Class 4	Firmicutes, plants, cyanobacteria	Bs, Mini-III	Single	Absent	Absent	Bs 23S rRNA maturation

Ec, *Escherichia coli*; Dm, *Drosophila melanogaster*; Hs, *Homo sapiens*; Bs, *Bacillus subtilis*.