Biochimica et Biophysica Acta xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Review

Intracellular ribonucleases involved in transcript processing and decay: Precision tools for RNA☆

Cecília Maria Arraiano [a], Fabienne Mauxion [b], Sandra Cristina Viegas [a], Rute Gonçalves Matos [a], Bertrand Séraphin [b,*]

[a] Control of Gene Expression laboratory, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República, 2780-1579 Oeiras, Portugal
[b] Equipe Labellisée La Ligue, Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Centre National de Recherche Scientifique (CNRS) UMR 7104/Institut National de Santé et de Recherche Médicale (INSERM) U964/Université de Strasbourg, 67404 Illkirch, France

ARTICLE INFO

Article history:
Received 11 December 2012
Received in revised form 19 March 2013
Accepted 22 March 2013
Available online xxxx

Keywords:
Ribonucleases
Protein domains
Protein families
RNA decay
RNA processing
Structure

ABSTRACT

In order to adapt to changing environmental conditions and regulate intracellular events such as division, cells are constantly producing new RNAs while discarding old or defective transcripts. These functions require the coordination of numerous ribonucleases that precisely cleave and trim newly made transcripts to produce functional molecules, and rapidly destroy unnecessary cellular RNAs. In recent years our knowledge of the nature, functions and structures of these enzymes in bacteria, archaea and eukaryotes has dramatically expanded. We present here a synthetic overview of the recent development in this dynamic area which has seen the identification of many new endoribonucleases and exoribonucleases. Moreover, the increasing pace at which the structures of these enzymes, or of their catalytic domains, have been solved has provided atomic level detail into their mechanisms of action. Based on sequence conservation and structural data, these proteins have been grouped into families, some of which contain only ribonuclease members, others including a variety of nucleolytic enzymes that act upon DNA and/or RNA. At the other extreme some ribonucleases belong to families of proteins involved in a wide variety of enzymatic reactions. Functional characterization of these fascinating enzymes has provided evidence for the extreme diversity of their biological functions that include, for example, removal of poly(A) tails (deadenylation) or poly(U) tails from eukaryotic RNAs, processing of tRNA and mRNA 3′ ends, maturation of rRNAs and destruction of unnecessary mRNAs. This article is part of a Special Issue entitled: RNA Decay mechanisms.

1. Introduction

RNA shares with DNA many physico-chemical characteristics yet two particular properties give RNA the ability to perform very specific functions in cells. The first is the presence of a 2′-OH group on ribose that renders RNA molecules much more labile than DNA. The second is the absence of a complementary strand for most RNA molecules. The latter allows some transcripts to adopt unique secondary and three-dimensional conformations, allowing structure-based functions (for example for tRNAs) or even endowing specific RNAs with catalytic properties (ribosome, RNase P, self-splicing introns and potentially even the spliceosome). However, a good number of RNA molecules in cells, particularly mRNAs, seem to be structured only to a limited extent and this folding is possibly heterogeneous, being locally modulated by the binding of a myriad of proteins. The mostly single stranded nature of RNA species makes them particularly good substrates for cleavage reactions. In contrast to DNA, these events are usually not repaired and thus lead to the irreversible degradation of the RNA transcript. The generally accessible structure and chemically reactive nature of RNA make this molecule well suited to function as a transient messenger or to serve as a substrate for maturation reactions. Yet, at the same time, cells must precisely control the various enzymatic activities (by the broadly named (ribo)nucleases) involved in RNA degradation and processing, to avoid undesired elimination of functionally important molecules.

The intrinsic unstable nature of RNA and the presence of numerous sources of non-specific contaminating activities were considerable obstacles to the identification of ribonucleases physiologically involved in the intracellular degradation and processing of transcripts. Over the years, however, many of these enzymes have been described and characterized, first from bacteria and more recently from archaea and eukaryotes. These enzymes were first described according to the type of reaction catalyzed: endoribonucleases if they cleave RNA molecules internally, or exoribonucleases if they attack RNAs from one of their extremities. Additional characteristics included the ability of these enzymes to cleave single and/or double stranded RNA, the nature of the products released, the specificity of the enzyme for substrates of defined shape (e.g., tRNA) and/or

☆ This article is part of a Special Issue entitled: RNA Decay mechanisms.

* Corresponding author. Tel.: +33 3 88 65 33 36; fax: +33 3 88 65 33 37.
E-mail address: seraphin@igbmc.fr (B. Séraphin).

1874-9399/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbagrm.2013.03.009

sequences, their ability to digest DNA besides RNA, and their processive or distributive action in cells. More recently, the identification of an ever-increasing number of ribonucleases combined with the unprecedented availability of protein sequences of unknown function derived from genome sequencing programs has allowed the grouping of these enzymes in defined families [1]. This further revealed the conservation of some of these factors across distant phyla as well as the evolution of some members, including duplication of enzymes, and acquisition or loss of particular properties. While the number and diversity of the currently known ribonucleases preclude an exhaustive presentation of this area, we propose to present here an overview of the major families of enzymes involved in the degradation and processing of cellular RNAs (summarized in Tables 1–3).

2. Exoribonucleases

2.1. DEDD exoribonucleases

DEDD ribonucleases belong to a large superfamily of proteins that has ramifications well beyond RNA degradative enzymes to include proofreading domains of polymerases and some deoxyribonucleases [1]. These proteins are all 3′ to 5′ exonucleases that generate 3′ OH and 5′ phosphate extremities, and are present in bacteria and eukaryotes. The structures of several members of this family have been determined (Fig. 1, e.g., [2–7]). They are organized around a 5-stranded β-sheet that is surrounded by α-helices and sometimes additional β-strands (Fig. 1). Beside ribonucleases and DNA exonucleases, this fold is found in other proteins, which, interestingly, have all functions related to nucleic acids (for example transposases and recombinases) [1]. The active site is formed by aspartates and glutamates, which are in the origin of the designation of this exonuclease subfamily. These residues, located on the surface of the central β-strand and in surrounding structural elements, coordinate the two metal ions required for catalysis [8]. Less-well conserved nearby amino acids often characteristic of particular subsets of enzymes, form the substrate binding pocket. While some members of this family only contain a DEDD module, some proteins contain additional domain(s) appended to, or even inserted into, it (Fig. 1).

One of the first characterized DEDD ribonucleases was *E. coli* RNase D. It was initially discovered due to its action over denatured and damaged tRNAs, however it can also act on tRNA precursors, 5S rRNA and other small structured RNAs [9,10]. RNase D is composed by one C-terminal DEDD domain (responsible for catalysis) and two N-terminal HRDC domains (Fig. 1A, [5]), which confer a distinct structure when compared to the other bacterial members of this family. *E. coli* RNase T is also a member of DEDD family. It is a single-stranded specific exoribonuclease, which also has a DNA exonuclease activity [11]. In order to be active, RNase T needs to form a dimer, adopting an architecture very similar to oligoribonuclease [12] (see below). RNase T is also involved in the 3′ maturation of some stable transcripts including ribosomal RNAs as well as in the degradation of tRNAs [13]. Proteins related to *E. coli* RNase D and RNase T are present in some, but not all, bacterial genomes sequenced, suggesting that equivalent functions may be performed by different enzymes in other species.

A particular member of the DEDD nuclease family is oligoribonuclease (Orn). It has the unique ability to degrade short oligonucleotides leftover by other nucleases into mononucleotides [14]. In the absence of Orn, 2- to 5-nucleotides-long RNA fragments accumulate, a situation deleterious for cells. This enzyme performs thus the final step of RNA degradation. In *E. coli*, oligoribonuclease is encoded by the *orn* gene and is a homodimeric protein with 3′ to 5′ processive activity (Fig. 1B, [15]). When the dimer is formed, the two DEDD domains appear to complement each other: one monomer binds to the substrate and leads it into the catalytic cavity of the second monomer [5]. Orn acts specifically on single-stranded RNA substrates. However, in high concentrations it is able to degrade short DNA oligonucleotides. Its activity is inhibited in the presence of 3′-phosphoadenosine 5′-phosphate (pAp), a nucleotide produced in prokaryotes and eukaryotes during the process of sulfur assimilation [16]. From all the exoribonucleases found in *E. coli*, Orn is the only one that has an essential function [14]. However, this is not a conserved property since in *Streptomyces griseus* and *S. coelicolor* Orn is not essential for growth even though it is crucial for the formation of aerial hyphae and to complete the life cycle [17]. In *Pseudomonas aeruginosa* Orn is also not essential and the 2 to 4 nucleotides RNA fragments accumulating in a mutant were shown to function as primers for transcription initiation [18]. Oligoribonucleases are found in Proteobacteria (β and γ divisions) and

Table 1
Exoribonucleases.

Family	Some prokaryotic members	Some eukaryotic members	Catalytic mechanism	Comments
DEDD	RNase D RNase T Orn	Pop2/Caf1 Pan2 PARN Rrp6 Rex2–4, Eri1/3′hexo/Snipper	Hydrolytic	3′ to 5′ activity Some members have sequence specificity (deadenylases) Family members can be processive or distributive Distant family members include DNases and polymerases
RNase II/ RNB	RNase II RNase R	Dis3 (Rrp44)/Dis3L1/Tazman Dis3L2/SOV Dss1 Ssd1	Hydrolytic	These enzymes are processive in the 3′ to 5′ direction Distributed by all domains of life
PDX	RNase PH PNPase Archeal exosomes	Plant Rrp41	Phosphorolytic	One or two PDX domains per protein Associate to form ring structures containing 6 PDX domains Many PDX domains are catalytically inactive, particularly in eukaryotes (Rrp41–43, 45, 46, Mtr3)
EEP	–	Ccr4 Nocturnin Ngl1–3	Hydrolytic	3′ to 5′ activity Some members have sequence specificity (deadenylases) Distant family members include DNases and phosphatases
DHH	NrnA NrnB	–		Also called nano RNases Decay polarity controversial Distant family members include DNases and phosphatases
Xrn1/Rat1	–	Xrn1/Pacman Rat1/Xrn2	Hydrolytic	5′ to 3′ decay Distantly structurally related to RNase H and FLAP nucleases

Please cite this article as: C.M. Arraiano, et al., Intracellular ribonucleases involved in transcript processing and decay: Precision tools for RNA, Biochim. Biophys. Acta (2013), http://dx.doi.org/10.1016/j.bbagrm.2013.03.009