Plant Science 262 (2017) 169–174

Contents lists available at ScienceDirect

Plant Science

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

Review article

Degradation of cytosolic ribosomes by autophagy-related pathways

Diane C. Bassham[a,*], Gustavo C. MacIntosh[b,*]

[a] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011, USA
[b] Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, IA 50011, USA

ARTICLE INFO

Keywords:
Autophagy
Degradation
Ribosome
rRNA
Vacuole

ABSTRACT

Ribosomes are essential molecular machines that require a large cellular investment, yet the mechanisms of their turnover are not well understood in any eukaryotic organism. Recent advances in Arabidopsis suggest that plants utilize selective mechanisms to transport rRNA or ribosomes to the vacuole, where rRNA is degraded and the breakdown products recycled to maintain cellular homeostasis. This review focuses on known mechanisms of rRNA turnover and explores unanswered questions on the specificity and pathways of ribosome turnover and the role of this process in maintenance of cellular homeostasis.

1. Introduction

As the machines for protein synthesis, ribosomes are essential to life. All cells dedicate a large proportion of their resources towards maintaining a ribosome number that can keep up with the translational needs of the organism. For example, in rapidly dividing yeast about 60% of total transcription is devoted to ribosomal RNA (rRNA), which encompasses approximately 80% of the total RNA content of a cell, while 50% of all RNA polymerase II transcription is allocated to ribosomal proteins [1].

Arabidopsis thaliana is among the eukaryotic organisms with the best characterized cytosolic ribosome proteomes [2]. Similar to other eukaryotes, the Arabidopsis genome contains hundreds of rRNA gene copies, clustered in two loci, which are either actively transcribed or differentially silenced through epigenetic mechanisms to regulate the effective rRNA dosage depending on the developmental stage and tissue [3]. These loci produce a primary transcript by Pol I that encodes the 18S, 5.8S, and 25S rRNAs. The remaining rRNA (5S) is transcribed independently by Pol III. The Arabidopsis genome also encodes 242 putatively functional r-protein genes encompassing 81 families of cytosolic r-proteins. About 60% of these gene products, representing 78 families, have been detected as ribosomal components in proteomics studies [2,4,5]. The cytosolic 80S ribosome is made of a large 60S subunit containing 5S, 5.8S, and 25/28S rRNAs and up to 47 different r-proteins and a small 40S subunit containing the 18S rRNA and up to 33 different r-proteins [2]. It has been suggested that differential incorporation of specific combinations of r-protein paralogs into the ribosome may result in ribosomes with distinct function [5].

The plant ribosomal components are well cataloged, yet the processes involved in plant ribosome biogenesis are not as well characterized as those of other model eukaryotes. However, a large number of ribosome processing factors that participate in pre-rRNA processing have been identified and a picture of the events that lead to mature ribosomes is starting to emerge. From this, it appears that plant ribosome biogenesis follows at least two alternative routes that combine characteristics of mammalian and yeast pre-rRNA processing pathways and include plant-specific features [reviewed in [6]]. The events that lead to the production of a functional translational apparatus on mRNA, and the selective translation of cytoplasmic mRNAs in response to developmental and environmental cues, have also been well-described for Arabidopsis and other plants [7–11].

A review of current literature shows that studies of the “life cycle” of eukaryotic cytoplasmic ribosomes in model and non-model organisms have mostly focused on biosynthesis and processing of their components, maturation, transport, and recruitment of the translation complexes on mRNA. In contrast, the last stage of their life, turnover of the ribosomal components, has received almost no attention. However, recent work in yeast, Arabidopsis and mammalian systems has begun to fill this knowledge gap.

2. Ribosome quality control mechanisms

Nuclear and cytoplasmic quality control mechanisms that eliminate nonfunctional ribosomes have been described. Introduction of mutations in rRNA in yeast led to the discovery of the Nonfunctional rRNA Decay (NRD) pathway that can detect and eliminate these defective ribosomes in the cytoplasm [12]. Yeast NRD is composed of two mechanistically distinct pathways, 18S NRD and 25S NRD. The 18S

Abbreviations: ATG, autophagy-related; ER, endoplasmic reticulum; NGD, no-go decay; NRD, nonfunctional rRNA decay

* Corresponding authors.
E-mail addresses: bassham@iastate.edu (D.C. Bassham), gustavo@iastate.edu (G.C. MacIntosh).

http://dx.doi.org/10.1016/j.plantsci.2017.05.008
Received 20 March 2017; Received in revised form 8 May 2017; Accepted 13 May 2017
Available online 19 May 2017
0168-9452/

NRD pathway, which occurs in P-bodies, shares mechanistic details with the mRNA quality control pathway known as No-go Decay (NGD). Both pathways are triggered by stalled ribosomes during translation and result in degradation of the RNA through the action of the ribonuclease Xrn1 and the cytoplasmic exosome, a 3′-5′ exonuclease complex [13,14]. In contrast, 25S NRD occurs outside of P-bodies, does not require ongoing translation and stalled ribosomes, and involves the exosome but not Xrn1 [14]. In addition, 25S NRD is mediated by ubiquitination of ribosome-associated proteins by an ubiquitin E3 ligase complex containing Mms1 and Rtt101, two proteins also associated with DNA repair mechanisms [15]. Misfolding and other ribosome assembly mistakes are also monitored in the nucleus by a TRAMP/exosome mechanism. Defective ribosome intermediates are surveyed in the nucleus by the TRAMP complex, which adds short polyA tails to nuclear RNAs that are then recognized by the nuclear exosome as substrates for degradation [16].

None of these rRNA quality control mechanisms have been studied in detail in plants. However, many of the proteins involved in these processes are conserved in plants, and some indirect evidence of their participation in rRNA processing exists. For example, 18S NRD components have been identified in Arabidopsis and evolutionary analyses suggest that NGD may be present in plants [17]. It has also been established that ribosome stalling leads to mRNA degradation in plants [see for example [18,19]], and since NGD and NRD are mechanistically related, it is possible that at least 18S NRD is also conserved. Mms1 and Rtt101 are part of an ubiquitin ligase family not present in other eukaryotes, yet they are functionally analogous to the CUL4 (DDB1) ubiquitin ligase that participates in DNA damage repair in plants and animals [20–22]. Interestingly, Arabidopsis CUL4 shuttles between the nucleus and the cytoplasm [22], suggesting that it could participate in a putative 25S NRD pathway.

The main ribonucleases involved in NRD, also shared with NGD and normal mRNA decay mechanisms, are conserved and well-characterized in Arabidopsis. Plants do not have an ortholog of Xrn1 but they have a cytoplasmic ortholog of the yeast nuclear 5′-3′ exonuclease Rat1/Xrn2 [23]. This enzyme, XRN4, is the functional analog of Xrn1 and contributes to mRNA decay in Arabidopsis [24,25]. XRN4, like yeast Xrn1, has been found associated with P-bodies [26]. The Arabidopsis exosome has also been well-characterized [27–31], although its role in NRD has not been studied. However, mutations affecting exosome components result in accumulation of rRNA precursors, indicating that the function of this complex in rRNA processing is conserved in plants [27,30]. The TRAMP component MTR4 is found in Arabidopsis, and *mtr4* mutants also accumulate rRNA precursors [32]. AtMTR4 co-purifies with exosome proteins, consistent with a role in exosome targeting, although a canonical TRAMP complex has not been identified in plants [33]. While the presence of the MTR4/exosome complex in plant nuclei could suggest that a ribosome quality control mechanism exists in these organelles, direct evidence of this process is lacking.

3. Autophagy-like mechanisms for ribosome turnover

Recent work from our laboratories have identified novel pathways of ribosome and/or rRNA turnover that seem to be independent of the quality control mechanisms described above, and implicate the autophagy machinery in a housekeeping pathway of normal rRNA turnover linked to maintenance of cellular homeostasis. Macroautophagy, most often simply called autophagy, is a ubiquitous degradation pathway in which substrates are enwrapped within double-membrane vesicles called autophagosomes and delivered to the vacuole, where they are broken down and the breakdown products recycled [34,35]. Autophagy is unusual among macromolecular degradation pathways in that it can degrade a wide range of different types of macromolecules due to the diversity of lytic enzymes present in vacuoles. It is also capable of degrading large structures in addition to individual macromolecules, including large complexes, organelles, and aggregates [36–40]. These properties provide enormous flexibility to the pathway in terms of the substrates that it can turn over and the conditions in which it can act. For example, whereas individual polypeptides can be degraded by the proteasome, under stress conditions proteins often aggregate and can be degraded by autophagy [41,42].

The activation and execution of the autophagy pathway depends on a set of genes termed autophagy-related (*ATG*) genes, originally identified through mutant screens in yeast [43–45]. Many of these genes are required for autophagosome formation, and Arabidopsis, maize and rice knockout mutations in *ATG* genes lead to defects in stress tolerance and early senescence, although mutants are developmentally similar to wild-type plants under normal growth conditions [46–51]. These phenotypes have led to the extensive study of autophagy under stress conditions, but its role in cell homeostasis under non-stress conditions is only just being revealed.

A pathway for degradation of mature ribosomes by autophagy, termed ribophagy, has been described in yeast [52]. Upon nutrient starvation, yeast ribosomes can be degraded non-selectively by the general autophagy pathway and also selectively via a ribophagy pathway that involves the ubiquitination of ribosomal subunits. The selective ribophagy pathway requires the classical ATG machinery and can degrade 40S and 60S subunits, although their mechanisms of selection are distinct and require different components [52–54]. Ribophagy mutants are sensitive to starvation, indicating that ribophagy is required for recycling of nutrients for survival during nutrient stress.

Although the ribophagy pathway described above appears to occur only in yeast, we have discovered recently that autophagy and/or autophagy-like pathways function in the degradation of ribosomal RNA in Arabidopsis [55,56]. This degradation requires the T2 ribonuclease RNS2, a widely-expressed and evolutionarily-conserved enzyme [57,58] that localizes to vacuoles/lysosomes [55,59,60]. Mutations in RNS2 or related ribonucleases lead to phenotypes in which rRNA accumulates inside the vacuole/lysosomes [55,60] and have been linked to human disease [61]. Unexpectedly, an Arabidopsis *rns2* mutant was found to have constitutive autophagy, in which numerous autophagosomes are formed even under normal growth conditions rather than only upon environmental stress exposure. This autophagy is dependent on the known autophagy genes *ATG5* and *ATG9* [56], indicating that it represents the classical and well-studied bulk autophagy pathway, and that autophagy may be involved in delivering rRNA to the vacuole for recycling.

The constitutive autophagy phenotype and increased half-life of rRNA of the *rns2* mutant, and the vacuolar localization of RNS2, led us to speculate that rRNA, and potentially whole ribosomes, may be delivered to the vacuole by autophagy for degradation [55,56]. We tested this hypothesis by generating double mutants between *rns2* and *atg5* or *atg9* and found that *rns2* mutants accumulate rRNA inside the vacuole, consistent with a role in rRNA degradation. A mutation in *atg5* blocked this accumulation, supporting a role for autophagy in delivering the rRNA to the vacuole. Surprisingly, mutation of *atg9* had no effect on vacuolar accumulation of rRNA in the *rns2* mutant, indicating that ATG9 is not required for rRNA delivery [56]. This result led us to rethink the hypothesis that rRNA is delivered to the vacuole by the classical macroautophagy pathway and to consider alternative pathways for vacuolar transport.

One trivial possibility is that ATG9 is not absolutely required for macroautophagy and that the *atg9* mutant is therefore not blocked in autophagic degradation. There is some support in the literature for this possibility, in that autophagic flux is not completely blocked in an Arabidopsis *atg9* mutant, despite it being an apparent null mutant, [62]. However, recent work has shown that ATG9 is required for early stages of autophagosome formation from the endoplasmic reticulum (ER), with extensive accumulation of autophagosome-related tubules remaining attached to the ER in *atg9* mutants, rather than being released as