Plant Science 223 (2014) 134–145

Contents lists available at ScienceDirect

Plant Science

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

Differential transcript accumulation and subcellular localization of Arabidopsis ribosomal proteins

Raghavendra P. Savada, Peta C. Bonham-Smith*

Department of Biology, University of Saskatchewan, Saskatoon, SK S7N 5E2, Canada

ARTICLE INFO

Article history:
Received 3 January 2014
Received in revised form 12 March 2014
Accepted 14 March 2014
Available online 20 March 2014

Keywords:
Arabidopsis
Ribosomal proteins
Differential expression
Nuclear/nucleolar localization
Nucleolar import

ABSTRACT

Arabidopsis cytoplasmic ribosomes are an assembly of four rRNAs and 81 ribosomal proteins (RPs). With only a single molecule of each RP incorporated into any given ribosome, an adequate level of each RP in the nucleolus is a prerequisite for efficient ribosome biogenesis. Using Genevestigator (microarray data analysis tool), we have studied transcript levels of 192 of the 254 Arabidopsis RP genes, as well as the sub-cellular localization of each of five two-member RP families, to identify the extent to which these two processes contribute to the nucleolar pool of RPs available for ribosome biogenesis. While transcript levels from different RP genes show up to a 300-fold difference across the RP population, this difference is drastically reduced to ~8-fold when considering RP gene families. Under various stimuli, while the transcript level for most RP genes remains unchanged some show a significantly increased or decreased level. Subcellular localization studies in tobacco not only showed differential targeting of RPs to the cytoplasm, nucleus and nucleolus, but also differential nucleolar import rates. This degree of variation in gene regulation and subcellular localization of RPs hints at the possibility of extra-ribosomal functions for some RP isoforms.

1. Introduction

Arabidopsis (*Arabidopsis thaliana*) ribosomes are comprised of four rRNAs (26S, 5.8S, 5S and 18S) and 81 ribosomal proteins (RPs). Although it is the rRNAs that perform the key peptidyl transferase reaction and mRNA decoding functions of the ribosome [1,2], RPs play critical roles in ribosome biogenesis and function. They are involved in rRNA processing and folding, subunit assembly and transport, stabilization of subunit structure, interactions of ribosomes with various translation factors, and folding and targeting of nascent polypeptides [2–6].

Regardless of the quantity produced of each RP, only a single molecule of each (except acidic proteins P0, P1, P2 and P3) is incorporated into any given ribosome [2,5,7], presumably necessitating an equimolar availability of each RP in the nucleolus to ensure efficient subunit assembly [8,9]. Ribosome biogenesis is an energy-intensive process. In a rapidly growing yeast cell, *rDNA* transcription accounts for 60% of total genomic transcription, and RP-mRNA splicing accounts for 90% of total mRNA splicing [10]. Consequently, RP synthesis for ribosome biogenesis needs to be tightly and coordinately regulated at various levels of gene expression. Production of a RP either in excess of, or less than its partners could be deleterious [8]. Haploinsufficiencies of some RPs leads to growth retardation and other developmental abnormalities in yeast [11], *Drosophila* [12], mammals [13,14] and plants [15–17].

Equimolar availability can be ensured by coordinated regulation of RP gene expression at the transcriptional and post-transcriptional (mRNA turn over and translation) levels. In yeast, where 59 of the 79 RPs (RP families L28 and plant-specific P3 are absent) are encoded by two-member gene families, coordinated regulation occurs primarily at the level of transcription [8–10,18]. However, such coordinated regulation results only in similar, but not identical total amounts of each RP mRNA being produced. In exponentially growing cultures, a difference in transcript levels of up to five-fold has been reported between the most and the least abundant RP mRNAs, although most are within a two-fold range [19].

In mammals, as a result of expression from only a single gene copy for most RPs, variations in RP mRNA abundance are kept within a fairly narrow range, with a few exceptions that appear to be cell type-specific [20–22]. Regulation of expression of RPs can also occur post-transcriptionally through modulated recruitment of RP transcripts to polysomes, largely mediated by 5′ TOP (Terminal Oligopyrimidine Tract) sequences in these transcripts

* Corresponding author. Tel.: +1 306 966 4274; fax: +1 306 966 4461.
E-mail address: peta.bonhams@usask.ca (P.C. Bonham-Smith).

http://dx.doi.org/10.1016/j.plantsci.2014.03.011
0168-9452/

[23–25]. Stimulated by an appropriate mitogenic or growth signal, phosphoinositide-3 (PI-3) kinase turns on the signaling cascade that eventually displaces a repressor protein bound to the TOP sequence in RP mRNAs, permitting the translational machinery to bind and translation to proceed [23,26]. Also, in both yeast and mammals, RPs produced in excess of biological demand, are targeted for 26S proteasome-mediated degradation [27–29].

Considering the equimolar presence of RPs in ribosomes, it would be expected that all RPs would be expressed in equal or near equal quantities. However, recent reports suggest that a number of individual RPs have wide-ranging extraribosomal roles in processes such as transcription, translation, mRNA processing, DNA repair, apoptosis and tumorigenesis [30–32], that would suggest variation in individual RP quantity. RPs can localize to the nucleolus for cytoplasmic ribosomal subunit assembly with rRNAs [33–35], mitochondria [36], plastids [37], the cell surface [38], can be secreted from the cell [39], or can accumulate in the cytoplasm and nucleoplasm [34,39–41], all of which suggest extraribosomal functions. Gene expression profiling under numerous experimental paradigms have clearly shown differential regulation of expression of individual (or cohorts of) RP genes. Analysis of gene expression patterns for 89 RP genes in six adult human tissues identified large variations in the expression of these genes within each of the considered tissues, with 13 of the genes showing differential expression across the six studied tissues [42]. Differential translation of RP mRNAs has also been documented during the maturation of human monocyte-derived dendritic cells, where the mRNAs for 12 large subunit (LSU) RPs were found to be disengaged from polysomes, indicating translational down regulation of these mRNAs [43].

In spite of differential expression of RP genes, some degree of equimolar availability of RPs in the nucleolus can be achieved by adjusting mRNA turnover, translation and/or localization rates of RPs. However, some RPs like RPL23a, are known to be involved in rRNA processing [44], and as such may be required in the nucleolus in higher quantities than RPs that are solely structural components of the ribosome. Furthermore, the rate at which different RPs are incorporated into the ribosome or the rate at which unassembled RPs are degraded will also impact their level of availability in the nucleolus.

Coordinated regulation of expression of RPs in plants, where RPs are encoded by multigene families, many of which are comprised of more than two active members (in Arabidopsis, RP gene families consist of two to seven active members), is much more complex than mammals. Members of many plant RP families exhibit differential expression in different tissues, developmental stages or in response to stress [45–47]. For instance, Arabidopsis *RPS5A* is strongly expressed in dividing cells, while its paralog *RPS5B* is predominantly expressed in differentiating cells [17]. *RPL11B* is highly active in proliferating tissues such as shoot and root apical meristems, whereas its paralog *RPL11A* is active in the root stele and in anthers [47]. In response to UV-B treatment, expression of Arabidopsis *RPL10C* is upregulated, while expression of *RPL10B* is downregulated [48]. Differential expression of RP isoforms, within a single tissue, has also been reported in *B. napus* [49]. While there has been some studies of the expression patterns of members within a number of RP families in Arabidopsis, there is little information pertaining to a coordinate regulation across RP families.

Using Genevestigator [50] to analyze Arabidopsis 22k microarray data, we have analyzed the extent of coordinate regulation of RP gene expression at both the individual RP gene and the RP gene family levels. We have studied the subcellular localization patterns of five two-member RP families; RPS3a, RPS8, RPL7a, RPL15 and RPL23a, as well as differences in the nucleolar import rates of RPS8A, RPL15A and RPL23aA.

2. Materials and methods

2.1. Plant material

Six-week-old plants of tobacco (*Nicotiana tabacum*), cultivar Petit Havana, grown in a growth chamber under a 23 °C/18 °C temperature regime and a 16 h/8 h photoperiod of ~170 μmol photons m^{-2} s^{-1} were used for all transient expression analyses.

2.2. RP transcript level analysis

To analyze the transcript levels of RPs at different developmental stages of Arabidopsis, GENEVESTIGATOR (https://www.genevestigator.com, October 2011), a database (normalized with RMA algorithm) and data mining interface for microarray data (Affymetrix GeneChip data), was used [50]. Values of RP transcript levels over different developmental stages and fold change in transcript level under various stimuli from ATH1: 22k high quality arrays in wild type Columbia-0 genetic background were used. All RP genes where the transcript level did not exceed the background level in at least 50% of the arrays were excluded from this analysis.

2.3. Fluorescent fusion protein constructs

The ORFs of various RPs without stop codons were amplified by RT-PCR, wherein for *RPL23aA* and *-B*, total RNA [35] and for *RPS3aA* and *-B*, *RPS8A* and *-B*, *RPL7aA* and *-B*, and *RPL15A* and *-B*, cDNA clones obtained from the Arabidopsis Biological Resource Center (ABRC; Supplemental Table 1) were used as templates. The binary vector pGREENI0029 [51], modified by the addition of a 2XCaMV 35S promoter-GST-EGFP-nos terminator cassette in the unique *Apa*I/*Not*I restriction sites [35] was used for all fusion protein constructs. All RP ORFs were cloned into unique *Eco*RI/*Bam*HI sites between the 35S promoter and GST linker, resulting in pGREENI0029-*35S-RP ORFs-GST-EGFP-nos* constructs. Although small proteins can localize to the nucleus by diffusion, preferential and timely localization of critical nuclear proteins like ribosomal proteins may rely on active transport [52]. A GST linker was added to the construct to increase the mass of the fusion protein beyond the exclusion limit of the nuclear pore complex (>60 kD) [35].

2.4. Transient expression in tobacco and confocal microscopy

Agrobacterium tumefaciens, LBA4404 [53] was cotransformed with pGREEN constructs and the binary vector pSOUP, that provides the *in trans* replication function for pGREEN [51]. Tobacco leaf epidermal cells were infiltrated with cultures of transformed *A. tumefaciens* (OD_{600} – 0.2) [54] and live cell imaging was carried out 72 h after infiltration using an inverted Zeiss LSM 510 META CLSM (Jena, Germany) with an Argon laser (488 nm) and 505–530 nm bandpass filter. Image processing was carried out using Zeiss LSM Image Browser and Adobe photoshop software (San Jose, CA, USA) and cytoplasmic, nuclear and nucleolar intensities of EGFP fusions were measured using imageJ (http://rsbweb.nih.gov/ij/). Statistical analysis (Student's *t*-test; two sample assuming unequal variance, single factor ANOVA and Pearson's correlation coefficient) was carried out using the Analysis ToolPak of Microsoft office 2007.

2.5. Fluorescence recovery after photobleaching (FRAP)

Due to the movement of nuclei/nucleoli within tobacco leaf epidermal cells, that could not be overcome by treatment with the actin depolymerization agent latruncilin B, FRAP assays were carried out manually. Prior to photobleaching, five images of EGFP fluorescence in the nucleolus were acquired. The complete area