

Seed-Stored mRNAs that Are Specifically Associated to Monosomes Are Translationally Regulated during Germination^{1[OPEN]}

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The life cycle of many organisms includes a quiescent stage, such as bacterial or fungal spores, insect larvae, or plant seeds. Common to these stages is their low water content and high survivability during harsh conditions. Upon rehydration, organisms need to reactivate metabolism and protein synthesis. Plant seeds contain many mRNAs that are transcribed during seed development. Translation of these mRNAs occurs during early seed germination, even before the requirement of transcription. Therefore, stored mRNAs are postulated to be important for germination. How these mRNAs are stored and protected during long-term storage is unknown. The aim of this study was to investigate how mRNAs are stored in dry seeds and whether they are indeed translated during seed germination. We investigated seed polysome profiles and the mRNAs and protein complexes that are associated with these ribosomes in seeds of the model organism *Arabidopsis* (*Arabidopsis thaliana*). We showed that most stored mRNAs are associated with monosomes in dry seeds; therefore, we focus on monosomes in this study. Seed ribosome complexes are associated with mRNA-binding proteins, stress granule, and P-body proteins, which suggests regulated packing of seed mRNAs. Interestingly, ~17% of the mRNAs that are specifically associated with monosomes are translationally up-regulated during seed germination. These mRNAs are transcribed during seed maturation, suggesting a role for this developmental stage in determining the translational fate of mRNAs during early germination.

Seeds represent a unique stage of the plant life cycle, characterized by a quiescent state with low metabolic activity compared with other developmental stages. Dry seeds are heterotrophic and contain all the components that are required for germination and seedling establishment until the seedling reaches the autotrophic

state and can photosynthesize. The seed is filled with bioactive molecules such as proteins and mRNAs. Seed-stored mRNAs can survive for years, which is surprising considering their short half-life times (minutes to hours) in metabolically active cells (Narsai et al., 2007). Seed-stored mRNAs accumulate during seed development (Sano et al., 2015) and retain their function during the dry storage until seed germination (Hughes and Galau, 1989; Comai and Harada, 1990; Galau et al., 1991). Thousands of stored mRNAs have been detected in seeds by microarray analysis in *Arabidopsis* (*Arabidopsis thaliana*), rice (*Oryza sativa*), wheat (*Triticum* sp.), and barley (*Hordeum vulgare*; Nakabayashi et al., 2005; Sreenivasulu et al., 2008; Howell et al., 2009; Yu et al., 2014; Sano et al., 2015). These stored mRNAs are selectively translated during seed germination and post-germinative seedling growth (Sano et al., 2012; Galland et al., 2014; Bai et al., 2017). Translation of prior stored mRNAs is not unique to seeds. During mammalian oocyte-embryo transition, the first embryonic division occurs before the initiation of zygotic transcription (Tadros and Lipshitz, 2009). Similar events are also seen

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L.B. and J.H. conceived this project and together with B.B. designed all research. B.B. performed the experiments with assistance from J.H.G.C. B.B. analyzed the data with assistance from S.v.d.H. and T.A.H.P.A. under the supervision of L.B. and J.H. B.B., L.B., and J.H. wrote the article.

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in insects and fish during embryonic development, where stored maternal mRNAs are mobilized for either translation or degradation determined by mRNA modifications such as methylation (Zalokar, 1976; Anderson and Lengyel, 1979; Evsikov and Marín de Evsikova, 2009; Fu et al., 2014; Zhao et al., 2017).

How mRNAs are protected during storage is unknown, but this is likely due to their association with RNA-binding proteins (RBPs; Dedow and Bailey-Serres, 2019). RBPs affect different aspects of mRNA metabolism including RNA biogenesis and processing, alternative splicing, polyadenylation, transport, stabilization, translation, and decay. RBPs hold specific RNA-binding domains such as the RNA recognition motif (RRM), the hnRNP K homology domain, or the DEAD box helicase domain (Lunde et al., 2007; Cléry et al., 2008; Valverde et al., 2008; Linder and Jankowsky, 2011). Binding of these proteins to sequence elements in mRNA can both promote and repress translation (Williams and Fox, 2003; Klimek-Tomczak et al., 2004; Zhang et al., 2017). Other sequence elements such as stop codons (Sunohara et al., 2004a), upstream open reading frames (uORFs; Wiese et al., 2004; Sachs and Geballe, 2006; Rahmani et al., 2009), and sequences encoding specific attenuator peptides (Sunohara et al., 2004b; Spevak et al., 2010) can trigger ribosome stalling and thus inhibit translation. RBPs can also interact directly with the ribosome and regulate translation (Carter et al., 2001; Merz et al., 2008; Francisco-Velilla et al., 2016). Ribosomes consist of proteins (ribosomal proteins) and ribosomal RNAs. Storage of ribosomes in quiescent cells has been documented in many different species and organs, including bacteria (Idriss and Halvorson, 1969; Chai et al., 2014), yeast (Waldron et al., 1977), sea urchin egg (Hultin, 1961), and the eggs of fruit flies (Kronja et al., 2014), and are necessary for reinitiation of translation and posttranscriptional processing related to stress response and developmental processes (Bailey-Serres et al., 2009). mRNAs and ribosomal proteins can also form higher order structures like processing-bodies and stress granules, which have also been related to the (temporal) storage of mRNA induced by environmental stimuli or developmental programs (Balagopal and Parker, 2009; Buchan and Parker, 2009; Decker and Parker, 2012).

The mechanisms by which mRNAs are stored in seeds are still unknown (Sajeev et al., 2019); however, the importance of these mRNAs has been suggested for quite some time (Rajjou et al., 2004; Nakabayashi et al., 2005; Kimura and Nambara, 2010). Previous work has revealed associations between mRNAs and protein complexes (Marcus and Feeley, 1964; Dure and Waters, 1965; Chen et al., 1968; Ihle and Dure, 1970; Beevers and Poulson, 1972; Poulson and Beevers, 1973; Hammett and Katterman, 1975; Ajtkhozhin et al., 1976; Harris and Dure, 1976); however, due to technical limitations at the time of publication, these studies failed to identify specific mRNAs. Here, we aimed to identify seed-stored mRNAs, their associated proteins, and their roles during seed germination. We show that

seed-stored mRNAs are associated with ribonucleic protein complexes and that a proportion of these mRNAs are translationally up-regulated during early seed germination, suggesting that they are translated during this important life cycle stage.

RESULTS

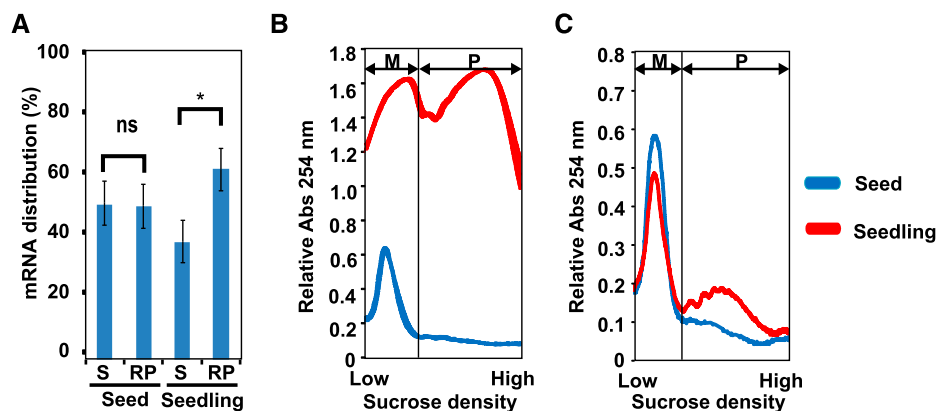
mRNAs Are Bound to Ribosomes in Both Dry Seeds and Seedlings

Seeds contain mRNAs that have accumulated during seed maturation; however, how these mRNAs survive storage has remained unclear. Using polysome profiling, we previously showed that dry seeds mainly contain monosomes when compared with polysomes (Bai et al., 2017). To determine the proportion of the total mRNA that is bound to these monosomes, total protein extracts were size-separated over a Suc cushion. mRNAs bound to large complexes, such as ribosomes, pass through the cushion while free RNAs are left in the supernatant. Total RNA and mRNAs were isolated from both the supernatant and the ribosome pellet, and the mRNA enrichment in these fractions was determined. Ribosome complexes were isolated from after-ripened dry seeds (hereafter referred to as seeds) and seedlings after 72 h imbibition. In seeds, there is an equal distribution of free and polysome-associated mRNA. In seedlings, a higher proportion of mRNA is associated with the polysome (Fig. 1A), which is in agreement with higher translational activity in seedlings. The complexes from the pellet were further separated by Suc gradient profiling. This confirmed that seeds mainly contain lower order ribosomes (ribosomal subunits and 80S ribosomes), whereas seedlings also contain polysomes (Fig. 1, B and C).

Specific mRNAs Are Bound to the Monosomes in Dry Seeds

We showed that dry seeds mainly contain monosomes (Fig. 1, B and C). To identify the mRNAs that are specifically associated with the monosome (M), these were compared with those of polysomes (P) in both seeds and seedlings using microarray analysis. Our gradient did not differentiate between ribosomal subunits and the 80S ribosome; however, because only the 80S ribosome binds mRNA we consider the mRNAs associated with this fraction to be monosome associated. Array probe signals were normalized and showed similar distribution in all of the hybridized samples (Supplemental Fig. S1, A–D). No obvious RNA degradation was observed in any of the samples (Supplemental Fig. S1E), and the sample preparation was robust based on the same signal distribution after normalization and high similarity between sample replicates ($r > 0.96$ for all comparisons and on average $r = 0.97$; Supplemental Fig. S2). Among the 27,827

Figure 1. mRNA distribution and polysome profiles of seeds and seedlings. A, mRNA distribution in seeds and seedlings [nonribosome fraction (S) and ribosome pellet (RP) fraction]. Mean \pm SD, **P*-value <0.05 (Student's *t* tests) are indicated. B and C, Polysome profiles of dry seeds and germinated seedlings from same dry weight (B) and similar ribosome loading (C; derived from Bai et al., 2017). Monosome (M) and polysome (P) regions in the polysome profile are indicated. Width of the line in (B) and (C) represents SD from the mean of 3 biological replicates (*n* = 3). ns, not significant.



genes investigated on the array, 17,189 were identified as present in either seeds or seedlings (Supplemental Table S1). Of these ~60% were present in both monosome and polysome fractions. By differential expression analysis between monosome- and polysome-associated transcripts, we identified genes to be significantly associated with either the monosome or polysome fraction (Fig. 2, A and B; Supplemental Table S2). Microarray analyses comparing samples that contain different amounts of mRNA are difficult, and they are not always corrected for by normalizing the data. This applies also to our data, especially for dry seeds, which hardly contain polysomes and therefore less mRNA in comparison with monosome samples. For the seedlings, we have equal amounts of mono- and polysomes and therefore do not expect problems related to data normalization. To validate the mRNA distribution in dry seeds, reverse transcription-quantitative PCR (RT-qPCR) analysis for the top ten monosome- and polysome-associated mRNAs was performed on the individual fractions of the Suc gradient (Fig. 2, C–E; Supplemental Table S3). The RT-qPCR results have been normalized to spikes that have been added to fractions before isolating the mRNA to allow the comparison of absolute mRNA levels in the two fractions (Fig. 2E). These analyses confirmed the specificity of the monosome-specific transcripts, as they were mainly present in the fractions containing the ribosomal subunits and the 80S ribosomes. The transcripts identified as polysome-specific in the microarray analyses appeared to be distributed across all fractions. Because we cannot confirm that the polysome-associated transcripts are specific, we will focus on the monosome-associated transcripts in dry seeds.

Common Sequence Characteristics for Monosome-Associated mRNAs

To further characterize the mRNAs specifically associated to the monosome, we calculated the monosome/polysome score for each mRNA. This is the log fold enrichment of the mRNAs in the monosome

fraction compared with the polysome fraction. Positive values indicate that the mRNA has a relatively higher association with monosomes (Heyer and Moore, 2016). In seeds, monosome-specific transcripts are generally shorter than transcripts that are associated with both monosomes and polysomes (Supplemental Fig. S3). This dominant length effect on monosome/polysome score was mainly attributed to the difference in length of coding sequence and 5' untranslated region (UTR) and to a lesser extent, by the 3' UTR (Supplemental Fig. S3). Sequence characteristics of monosome-specific transcripts were further investigated in each predefined transcript length group. This revealed that the guanine-cytosine (GC) content in the UTRs of the monosome-specific transcripts is low (Supplemental Fig. S4, A–D). GC content in the third position of the codon (GC3%) and effective number of codons (Nc) are normally used as indicators for codon usage bias. The monosome-specific transcripts were significantly higher in GC3 content, but showed lower Nc values (Supplemental Fig. S4, E–F), indicating that monosome-specific transcripts tend to have a biased codon usage and relatively high abundance (Supplemental Fig. S4G). This is in contrast with a higher codon usage bias related to a higher optimal frequency and mRNA abundance for the polysome-specific transcripts reported in yeast (Heyer and Moore, 2016). mRNA stability and intron number have been suggested to play a role in translation regulation (Oliveira and McCarthy, 1995; Nott et al., 2003). mRNA half-life times and intron numbers of monosome-specific transcripts were determined from a previous Arabidopsis study (Narsai et al., 2007). No significant differences in mRNA decay rate, half-life, and intron number were identified for mRNAs that are specifically associated with monosomes (Supplemental Fig. S5, A–D). In seedlings, we observed similar differences between the monosome- and polysome-associated transcripts when considering the length distribution and the other sequence features (including length distribution, GC content, mRNA stability, and intron numbers; Supplemental Figs. S6–S8). Thus, mRNAs associated with monosomes share distinct features from those associated with polysomes and this distinction is not dependent on the

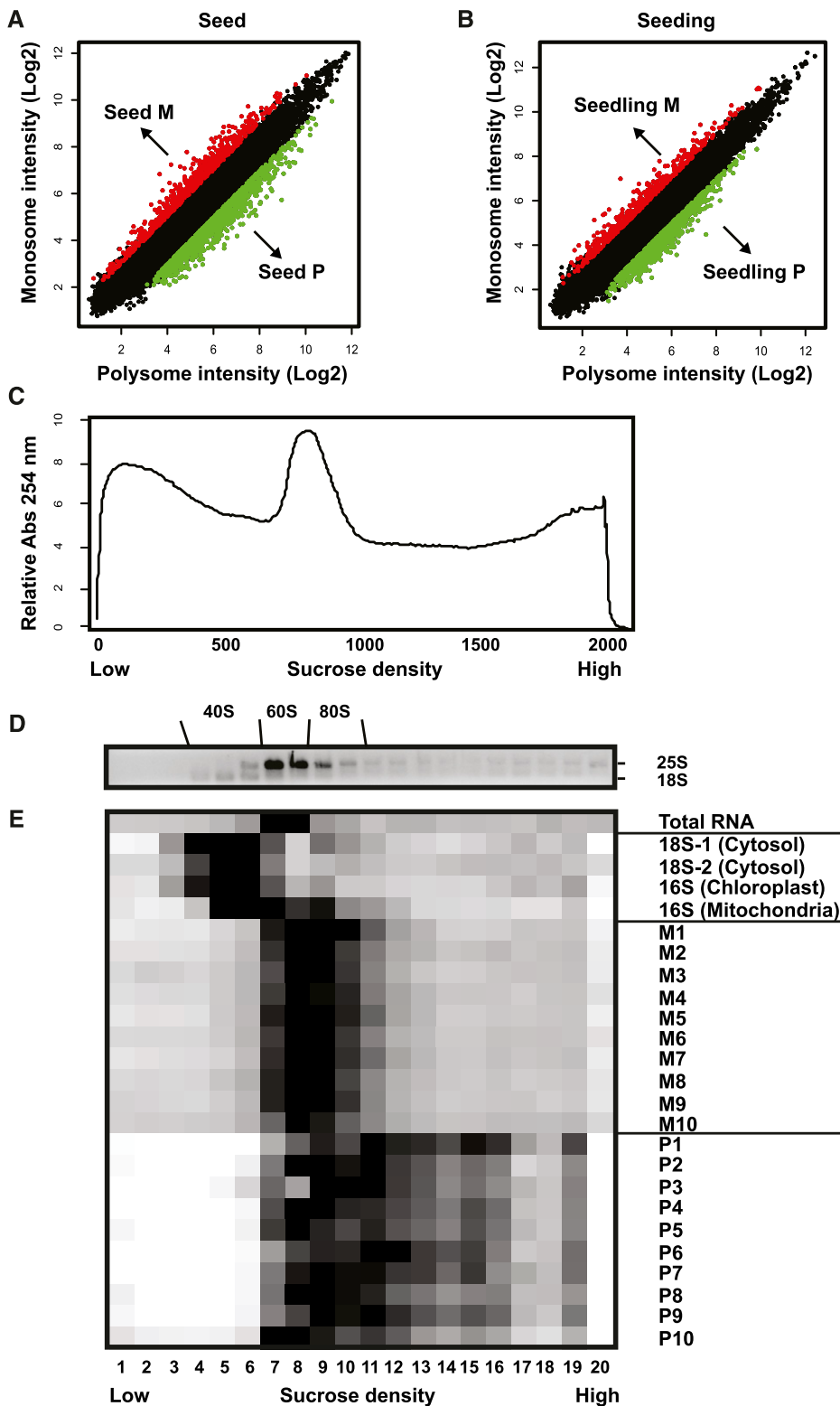


Figure 2. Comparison of monosome- and polysome-associated transcripts in seeds and seedlings. **A**, Intensity of mRNAs associated with the monosome versus the intensity of the same mRNAs associated with the polysome in dry seeds. Red and green dots indicate the monosome-specific and polysome-associated transcripts (fold change > 2, FDR < 0.05). **B**, Intensity of mRNAs associated with the monosome versus the intensity of the same mRNAs associated with the polysome in seedlings. Red and green dots indicate the monosome-specific and polysome-associated transcripts (fold change > 2, FDR < 0.05). **C** to **E**, Validation of mRNA distribution in dry seeds (**C**). Polysome profile of dry seeds is separated on a Suc gradient. The total gradient has been divided into 20 fractions [see numbers in (**E**)]. **D**, Ribosome fraction loaded on an agarose gel; 25S and 18S rRNA bands are indicated. **E**, Total RNA quantification (Nanodrop) and the relative intensity of 18S cytosolic rRNA (two primer pairs used), 16S plastidial rRNA, 16S mitochondrial rRNA, and the top 10 mRNAs associated with monosome (M) and polysome (P) as revealed from the microarray analyses. The relative intensity of each gene is based on RT-qPCR quantification, which is z-score normalized based on the intensity in all fractions and presented by the grayscale.

difference in translational status in dry seeds and seedlings.

Distinct Sequence Structures and Motifs Distinguish Monosome-Associated Transcripts

In our search of features that distinguish monosome-specific mRNAs from the general ribonucleic-protein-associated transcripts in seeds, sequence structure scores experimentally derived by Li et al. (2012) were evaluated for our dataset. High structure scores indicate more double stranded (ds) than single

stranded (ss) RNA at a certain position in a transcript and vice versa for low structure scores. Average structure scores were plotted over the 5'UTR, coding DNA sequence (CDS), and 3'UTR for monosome- and polysome-associated transcripts in both seeds and seedlings (Fig. 3A; Supplemental Fig.e S9A) and compared with the average score of all genes present on the array (background). In seeds, monosome-specific transcripts exhibited a strong reduction in secondary structure especially in 5'UTR and CDS region. This pattern is specific for monosome-associated transcripts and is not biased by the short length of these transcripts or their rather high expression in dry seeds

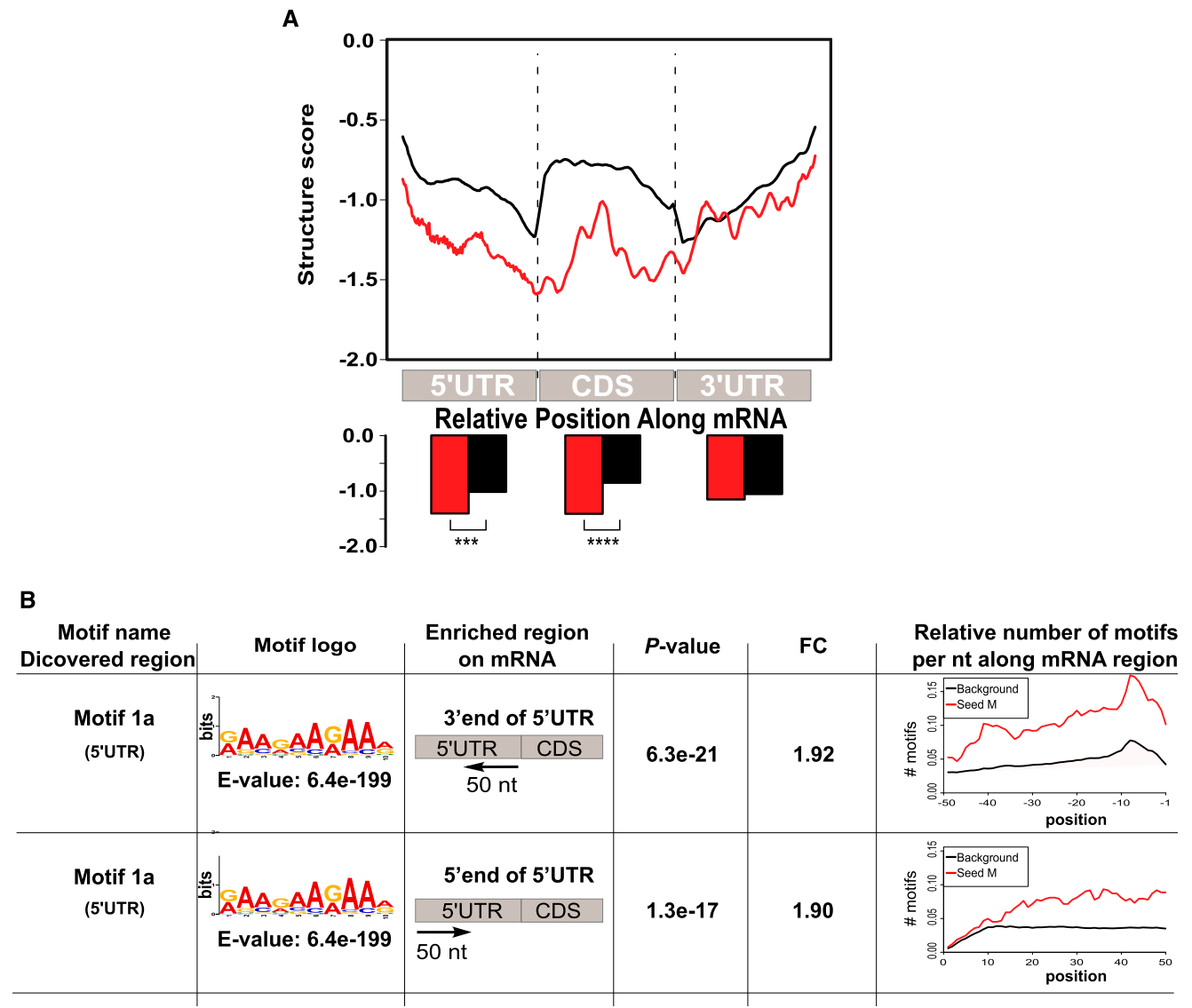


Figure 3. Secondary structure and motifs identified in transcripts specifically associated with monosomes in seeds. A, Secondary structure of monosome-specific transcripts (red line) in seeds. The black line represents the structure score of all mRNAs expressed in the experiment (background). Significant levels are indicated by *** $P < E-50$ and **** $P < E-100$. B, Motifs identified as enriched in mRNAs associated to seed monosome. Motif name, discovered sequence region, motif logo, enriched region, P -value (Fisher exact), and fold enrichment (FC) are compared with the background set and absolute motif position (position) along the transcripts with relative number of motifs (# motifs) per nt along the mRNA in the specified regions are indicated.

(Supplemental Fig. S10). In addition, we identified a motif with the consensus GAAGAAGAA (motif 1a), which is significantly overrepresented in the both 3' and 5' end of the 5'UTR and underrepresented in the general seed ribosome-associated transcripts (Fig. 3B). This strongly enriched motif might play a role in the recruitment of specific RNA-binding proteins.

The Monosome Enriched Proteome Contains mRNA Storage and Processing Related Proteins

We have shown that specific mRNAs are bound to the monosome complex in dry seeds. To investigate these ribosome protein complexes, high-throughput liquid chromatography–mass spectrometry (LC-MS) was applied to both the monosome- and polysome-enriched fractions. In total, 1,398 proteins were identified as present in the ribosome-enriched proteome in at least one fraction (monosome or polysome) in all three replicates (Supplemental Table S4). When the proteins from the monosome and polysome fractions were compared, 280 and 269 proteins were identified that were enriched in the monosome and polysome fractions, respectively (Supplemental Table S4). Monosome fractions were enriched for proteins involved in redox balance, translation factors, and protein with chaperon activity such as late-embryogenesis abundant proteins (LEAs), dehydrin, heat shock proteins, chaperonins, seed storage protein family such as cruciferins and RmlC-like cupins, and ribosomal proteins (Supplemental Table S5). Polysome fractions were high in abundance for proteins related to metabolism, mRNA splicing factors [e.g. Ser/Arg-rich proteins 33 (SR33), SR30] and histone-associated factors, and seed storage albumins and lipid transfer proteins (Supplemental Table S5). In the polysome enriched fractions, we identified several protein classes, such as splicing factors and histones, that are not related to the ribosome necessarily. These proteins were previously shown to be associated with ribosomes (Chibani et al., 2006; Sykes et al., 2010; Fuchs et al., 2011; Aviner et al., 2017). Nevertheless, we cannot exclude the possibility that these proteins are an artifact from the isolation method, since size-based fractionation does not exclude coelution of other large protein complexes.

In total, 135 ribosomal proteins were identified in the seed ribosome-enriched proteome. This accounted for 55.8% of the recently annotated 242 ribosomal proteins (Hummel et al., 2015). The abundance of the ribosomal proteins was higher in the monosome fraction when compared with the polysome fraction, as was revealed both at the protein and peptide level (Supplemental Tables S6 and S7). This is consistent with the dry seed polysome profile that mainly consists of a monosome peak (Fig. 1, B and C). The seed ribosome composition is homogenous in the monosome and polysome, as revealed by comparing the peptide signal of specific ribosome proteins to the total ribosome peptide signal (Supplemental Table S7).

In addition, abundant RBPs and components from two types of cytoplasmic foci, stress granules (SG) and P-bodies, were identified (Table 1). Annotation of these proteins was based on their mammalian homologs (Table 2). Stress granules are thought to function in temporary mRNA storage and translation initiation, whereas the function of P-bodies, which is currently heavily debated, lays either in mRNA degradation and translational repression or mRNA storage (Weber et al., 2008; Chantarachot and Bailey-Serres, 2018; Standart and Weil, 2018).

Monosome mRNAs Prime Seeds for Germination

In both seeds and seedlings, monosome-specific transcripts were identified (643 in seeds and 555 in seedlings), of which only about 10% were shared between the two developmental stages (Fig. 4A). Previously, we have reported extensive translational regulation during early germination (Bai et al., 2017). Interestingly, 17% of the monosome-specific transcripts in dry seeds (112/643) were found to be specifically translationally up-regulated during seed germination, while transcripts that are associated with both monosomes and polysomes are translationally down-regulated during early seed germination (Fig. 4B; Supplemental Table S2). Notably no monosome-specific transcripts are down-regulated and none of other seed monosome- and polysome-associated transcripts are up-regulated during germination (Fig. 4B). This indicates that mRNAs with a high translation potential during early germination are stored in association with monosomes specially, whereas general ribosome-associated mRNAs are possibly leftover transcripts of seed maturation, suggesting that mRNAs are primed during seed maturation for a specific fate during germination (Fig. 4C). Transcripts that are identified both in association with the monosome in dry seeds and that are translationally up-regulated during early seed germination encode genes that are involved in water deprivation and cell cycle arrest (Supplemental Table S8). These transcripts are likely to play a role in the initial stages of seed germination by promoting early seed hydration and cell cycle activation.

DISCUSSION

Dry seeds contain stored mRNAs that in some species (such as date palm and lotus) tolerate dry storage for more than a thousand of years without losing the ability to germinate and grow (Shenmiller et al., 1995; Sallon et al., 2008). How the mRNAs are stored, protected, and eventually support seed germination and seedling growth have remained largely elusive (Sajeev et al., 2019). The work here provides insight into the role of the seed-stored mRNAs in Arabidopsis and reveals that mRNAs are primed during seed maturation for a later fate during germination (Fig. 4).

We show that ~50% of seed-stored mRNAs are bound to ribosomes, mostly monosomes (Fig. 1, A and B). Seed-stored monosomes are distinct from those of

yeast and mammalian inactive monosomes because the dry seed is translationally quiescent, whereas yeast and mammalian cells are actively translating (Heyer and

Table 1. Proteins identified in the seed monosome and polysome fractions that based on their annotation have been associated with RNA binding.

For each RBP, the protein the Arabidopsis Genome Initiative (AGI) number, protein symbol, and the protein name are indicated. Proteins with symbols undefined are indicated by a dash line.

AGI Protein	Protein Symbol	Name
Monosome		
AT1G59700	ATGSTU16	GST 16
AT1G78380	ATGSTU19	GST 19
AT1G69410	ELF5A-3	Eukaryotic Translation Initiator 5A-3
AT2G30860	ATGSTF9	GST Phi 9
AT2G47730	ATGSTF8	GST 8
AT3G55620	EMB1624	Embryo Defective 1624
AT5G11200	DEAD/DEAH BOX15	Dead-Box ATP-Dependent RNA Helicase 15
AT2G29450	ATGSTU5	GST TAU 5
AT1G18070	EF1A	Translation Elongation Factor 1A
AT1G57720	EF1B	Translation Elongation Factor 1B
AT1G68010	HRP	Hydroxypyruvate Reductase
AT2G21660	ATGRP7	Gly Rich Protein 7
AT2G33410	RRM/RBD/RNP	RNA-Binding (RRM/RBD/RNP Motifs) Family Protein
AT5G07350	TUDOR1	Tudor-Sn Protein 1
AT5G54900	ATRBPA45A	RNA-Binding Protein 45A
AT5G61780	TUDOR2	Tudor-Sn Protein 2
AT5G66190	FNR1	Ferredoxin NADP Reductase 1
AT1G29880	—	Glycyl-tRNA Synthetase
AT5G16840	BPA1	Binding Partner of ACD11 1
Polysome		
AT1G80070	SUS2	Abnormal Suspensor 2
AT3G50670	U1-70K	U1 Small Nuclear Ribonucleoprotein 70 KDa
AT3G15590	TPR-LIKE	Pentatricopeptide Repeat-Containing Protein
AT5G41770	—	Crooked Neck Protein
AT3G05060	NOP56-LIKE	Probable Nucleolar Protein 5-2
AT5G60790	ATGCN1	General Control Non-Repressible 1
AT4G31180	IBI1	Impaired in BABA-Induced Disease Immunity 1
AT2G29140	APUM3	Pumilio 3
AT1G53280	DJ1B	Homolog of Animal DJ-1 Superfamily Protein
AT3G20250	APUM5	Pumilio 5
AT1G54270	EIF4A-2	Eukaryotic Initiation Factor 4A-2
AT1G64550	ATGCN3	General Control Non-Repressible 3
AT5G47010	LBA1	Regulator of Nonsense Transcripts 1 Homolog
AT2G33340	MAC3B	PremRNA-Processing Factor 19 Homolog 2
AT3G02720	DJ1D	Class I Gln Amidotransferase-like Superfamily Protein
AT3G02760	—	Class II aaRS and Biotin Synthetases Superfamily Protein
AT1G56110	NOP56	Homolog of Nucleolar Protein NOP56
AT5G53440	—	Zinc Finger CCCH Domain Protein
AT1G76810	EIF-2	Eukaryotic Translation Initiation Factor 2
AT1G55310	SR33	SC35-Like Splicing Factor 33
AT1G07360	—	Zinc Finger CCCH Domain-Containing Protein 4
AT1G32790	CID11	CTC-Interacting Domain 11
AT1G67680	—	SRP72 RNA-Binding
AT2G18510	EMB2444	Embryo Defective 2444
AT2G39780	RNS2	RNase 2
AT2G40660	—	OB-Fold-Like Protein
AT3G14450	CID9	CTC-Interacting Domain 9
AT3G55460	SCL30	SC35-Like Splicing Factor 30
AT3G61860	RSP31	Arg/Ser-Rich-Splicing Factor Factor Rsp31
AT5G52040	ATRSP41	Arg/Ser-Rich-Splicing Factor Factor Rsp41
AT1G76010	ALBAT1	Alba DNA/RNA-Binding Protein
AT2G37340	RSZ33	Arg/Ser-Rich Zinc Knuckle-Containing Protein 33
AT3G26420	ATRZ-1A	RNA-Binding Gly-Rich Protein B2

Table 2. Putative SG and P-body (PB) components identified in association with the seed monosome and polysome (see Supplemental Table 2).

For each putative SG or PB protein, the AGI number is indicated. Furthermore, the table provides information on whether the protein was associated with monosomes, polysomes, or both; which component (SG or PB) it putatively corresponds to; the protein symbol and name; the organism in which the homolog was identified; the name in this organism; and the reference.

AGI	Ribosome Association	Granule Type	Protein Symbol	Protein Name	Organism	Homolog	Reference
AT2G33410	Monosome	SG/PB	RRM/RBD/RNP	RNA-binding (RRM/RBD/RNP motifs) family protein	Mammals	Musashi	Kawahara et al., 2008
AT5G10450	Monosome	SG	GRF6	14-3-3-Like Protein Gf14 Lambda	Mammals	14-3-3 protein	Stoecklin et al., 2004
AT5G16050	Monosome	SG	GRF5	14-3-3-Like Protein Gf14 Upsilon	Mammals	14-3-3 protein	Stoecklin et al., 2004
AT1G54270	Polysome	SG	EIF4A-2	Eukaryotic Initiation Factor 4A-2	Mammals	EIF4A	Kedersha et al., 2000
AT2G17870	Polysome	SG	AT2G17870	Cold Shock Domain Protein 3	Mammals	Lin28	Balzer and Moss, 2007; Polesskaya et al., 2007
AT2G29140	Polysome	SG	APUM3	Pumilio 3	Mammals	PUM1/2	Vessey et al., 2006
AT3G20250	Polysome	SG	APUM5	Pumilio 5	Mammals	PUM1/2	Vessey et al., 2006
AT4G36020	Polysome	SG/PB	CSDP1	Cold Shock Domain Protein 1	Mammals	Lin28	Balzer and Moss, 2007; Polesskaya et al., 2007
AT5G47010	Polysome	SG/PB	LBA1/UPF1	Low-Level Beta-Amylase 1	Arabidopsis	UPF1	Arciga-Reyes et al., 2006
AT1G02080	Polysome	PB	—	CCR4-Not Complex Component, Not1	Mammals, yeast	CCR4	Sheth and Parker, 2003; Andrei et al., 2005
AT1G03790	Polysome	PB	SOM/TZF4	Zinc Finger CCCH Domain-Containing Protein 2	Arabidopsis	TZF4	Bogamuwa and Jang, 2013
AT1G76810	Polysome	PB	EIF-2	Eukaryotic Translation Initiation Factor 2	Mammals	EIF-2	Kimball et al., 2003
AT2G17870	Polysome	PB	CSP3	Cold Shock Domain Protein 3	Mammals	Lin28	Balzer and Moss, 2007; Polesskaya et al., 2007
AT1G14170	Monosome and Polysome	SG/PB	—	RNA-Binding KH Domain-Containing Protein	Mammals	PCBP2	Fujimura et al., 2008
AT1G48410	Monosome and Polysome	SG/PB	AGO1	Protein Argonaute	Arabidopsis	AGO1	Morel et al., 2002
AT2G21060	Monosome and Polysome	SG/PB	ATGRP2B	Gly-Rich Protein 2B	Mammals	Lin28	Balzer and Moss, 2007; Polesskaya et al., 2007
AT3G13300	Monosome and Polysome	PB	VCS	Varicose	Arabidopsis	VCS	Goeres et al., 2007; Xu and Chua, 2009
AT1G22300	Monosome and Polysome	SG	GRF10	14-3-3-Like Protein Gf14 Epsilon	Mammals	14-3-3 protein	Stoecklin et al., 2004
AT1G54080	Monosome and Polysome	SG	UBP1A	Oligouridyate-Binding Protein 1A	Mammals, yeast	TIA-1	Kedersha et al., 1999; Buchan and Parker, 2009
AT1G56340	Monosome and Polysome	SG	CRT1	Calreticulin-1	Mammals	Calreticulin	Decca et al., 2007
AT1G78300	Monosome and Polysome	SG	GRF2	14-3-3-Like Protein Gf14 Omega	Mammals	14-3-3 protein	Stoecklin et al., 2004
AT2G27040	Monosome and Polysome	SG	AGO4	Argonaute 4	Mammals	AGO2	Leung et al., 2006
AT4G09000	Monosome and Polysome	SG	GRF1	14-3-3-Like Protein Gf14 Chi	Mammals	14-3-3 protein	Stoecklin et al., 2004
AT5G44500	Monosome and Polysome	SG	—	Small Nuclear Ribonucleoprotein Family Protein	Mammals	SMN	Béchéde et al., 1999; Hua and Zhou, 2004; Liu and Gall, 2007

(Table continues on following page.)

Table 2. (Continued from previous page.)

AGI	Ribosome Association	Granule Type	Protein Symbol	Protein Name	Organism	Homolog	Reference
AT5G47210	Monosome and Polysome	SG	—	Hyaluronan / mRNA Binding Family	Mammals	SERBP1	Goulet et al., 2008
AT5G65430	Monosome and Polysome	SG	GRF8	14-3-3-Like Protein Gf14 Kappa	Mammals	14-3-3 protein	Stoecklin et al., 2004
AT3G18130	Monosome and Polysome	SG	RACK1C	Receptor for Activated C Kinase 1C	Mammals	RACK1	Arimoto et al., 2008

Moore, 2016; Liu and Qian, 2016). Although the polysome peak is hardly visible in the dry seed polysome profiles (Fig. 1, B and C), their presence was revealed by the identification of mRNA and ribosomal proteins in the polysome fractions. GO categories of mRNAs that are associated with polysomes include “embryo development ending in seed dormancy,” “pollen development,” “flower development,” and “pollen germination,” which strongly suggests that these mRNAs are remnants of embryogenesis and seed maturation (Supplemental Table S9). Likely, these mRNAs are not required during seed germination and are therefore degraded upon imbibition. We cannot exclude the possibility that degradation of these mRNAs has already occurred in dry seeds. The RNA degradation plots of all arrays do not show obvious degradation patterns (Supplemental Fig. S1E); however, these plots lack information on the 5’ and 3’ ends of the mRNAs since the probes to which our RNA was hybridized are based on exon sequences. The degradation of mRNAs during seed imbibition is supported by the fact that many of these transcripts are translationally down-regulated during early seed germination (hydration translational shift; Supplemental Table S2). A protein that might be involved in the degradation of mRNAs that are not required for germination is VARICOSE. VARICOSE is associated with both monosomes and polysome and has been characterized as a P-body protein (Table 2), suggesting a role in mRNA degradation. The *varicose* mutant in Arabidopsis has a germination phenotype; Basbouss-Serhal et al. (2017) showed that *varicose* seeds accumulate transcripts whose degradation would prevent seed germination of the wild type, including those related to the GO categories “hormones,” “redox,” “cell wall,” and “transport.”

In contrast with this, we see that transcripts that are associated with monosomes in the dry state are translated (associated with polysomes) upon seed imbibition. Moreover, 17% of the mRNAs that are bound to monosomes specifically in seeds are translationally up-regulated during germination (Fig. 4, A and B; Supplemental Tables S2 and S8). This supports the hypothesis that translation of seed-stored mRNAs is important for seed germination (Harris and Dure, 1976; Aspart et al., 1984; Suzuki and Minamikawa, 1985; Beltrán-Peña et al., 1995; Nakabayashi et al., 2005). The identified monosome-specific transcripts include many that were identified as neosynthesized proteins during seed germination (Galland et al., 2014), such as *LEAs*, *HSPs*, and *ROTAMASE CYCLOPHILIN*. The proteins encoded by these transcripts are also found in the monosome-associated proteome (Supplemental Table S5) and might play roles as chaperons during early seed imbibition. The switch from the desiccated state to hydration during early seed imbibition is likely an important physiological transition as can be concluded from the high enrichment of genes with the GO category “response to water deprivation,” which are associated with monosomes and are translationally

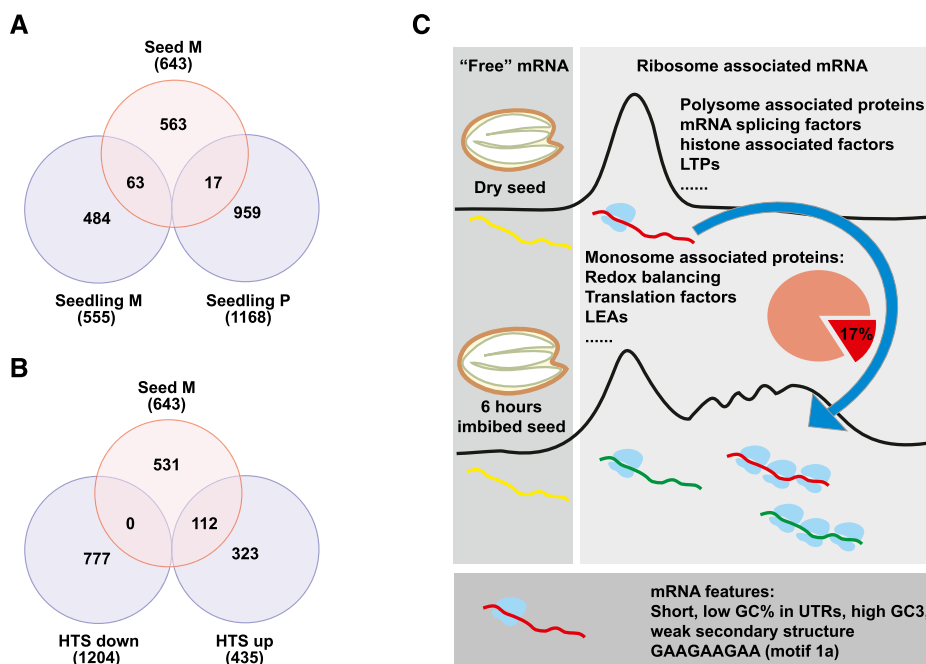


Figure 4. Monosome-specific transcripts in dry seeds are associated with polysomes during early seed germination. A, The comparison of monosome-specific transcripts in seeds (Seed M) to the seedling transcripts specific to monosome (Seedling M) and polysome (Seedling P). B, The comparison of transcripts specific to monosome in seeds (Seed M) to the transcripts translationally regulated during early seed germination [hydration translational shift (HTS)] identified by Bai et al. (2017). The number of transcripts is indicated between brackets [Fold change (FC) > 2, FDR < 0.05]. Lists with the transcripts can be found in Supplemental Table S2. C, A model presenting the fate of seed-stored transcripts. In dry seeds, approximately half of the mRNAs are associated with monosomes, whereas the other half of seed-stored mRNA is freely present in the cell. The seed monosome complex is associated with proteins involved in redox balance, translation factors, and proteins with chaperon activity such as LEAs, dehydrin, heat shock proteins, chaperonins, seed storage protein family such as cruciferins and RmlC-like cupins, and ribosomal proteins. Although not visible in the profile, dry seeds also contain polysomes, proteins associated with the polysome complex including proteins related to metabolism, mRNA splicing factors [e.g. Ser/Arg-rich proteins 33 (SR33) and SR30] and histone-associated factors, and seed storage albumins and lipid transfer proteins (LTPs). Seventeen percent of the seed monosome-specific mRNAs (indicated in red) are translationally up-regulated during early seed imbibition. The rest of the translationally up-regulated mRNAs are newly synthesized mRNAs (indicated in green).

up-regulated during early imbibition (Supplemental Table S8). Another GO category that is prominent among the monosome-specific transcripts is redox balance (Supplemental Table S9). Moreover, the proteome of the monosome fractions is enriched for proteins related to protection against oxidative stress (Supplemental Table S5). It is likely that these protective mechanisms safeguard mRNAs against oxidation during seed dry storage, such that they can be translated during seed germination. The SG and P-body proteins identified to be associated with monosomes and polysomes also contribute to this protection (Table 2). Currently, the cytoplasmic foci that are associated with these proteins in stress conditions have not been identified at the cellular level.

How specific mRNAs are targeted to monosome complexes and are specifically translated during imbibition is at present unknown; however, our data provide several indications. Transcript sequence features such as transcript length, GC content, and upstream open reading frames are key features for ribosome

association to either the monosome or the polysome; this is independent of the cellular translational status since these features are conserved between quiescent dry seeds and metabolically active seedlings, which each have their own specific set of transcripts (Supplemental Figs. S3–S8). The selectivity of specific transcripts for translational regulation possibly lies in the presence of specific sequence motifs in the selected mRNAs (Fig. 3). Interestingly, we identified reduced sequence structures and motifs specifically in the 5'UTR and before the start codon region. We hypothesize that specific RBPs bind these motifs and inhibit translation in seeds or inhibit the progression of ribosome from the translational initiation sites and thereby prime these mRNAs for translation during imbibition.

Our proteomic investigation of the monosome fractions supports this hypothesized role of RBPs. Detailed analyses of the ribosome protein complex revealed that next to ribosomal proteins, mRNA storage and processing granules could be essential components closely associated with ribosomes in the dry

seeds (Tables 1 and 2). These mRNA storage granules could transiently store mRNAs and restrict their translation either for translational reinitiation or degradation (Balagopal and Parker, 2009; Chantarachot and Bailey-Serres, 2018).

The monosomes described in our study have been identified by polysome profiling, a technique that is based on density centrifugation, which has been widely used to identify mRNAs that are associated with ribosomes (Juntawong and Bailey-Serres, 2012; Heyer and Moore, 2016; Bai et al., 2017). A shortcoming of this technique is that other protein complexes of similar size can coelute with the ribosomes; therefore, in theory, there could be false positives among the ribosome (monosome and polysome)-associated mRNAs identified. The coelution of other proteins cannot be excluded by the proteome analyses that we performed; however, for the monosome fraction we mostly identified proteins that have been described to be associated with ribosomes. The polysome fraction that is hardly visible in dry seeds did contain some proteins like splicing factors and histones, which might not be directly associated with the ribosome; this issue is also discussed in the “Results” section. An initial isolation of ribosomes based on immunoprecipitation, a technique also known as translating ribosome affinity purification (Zanetti et al., 2005; Reynoso et al., 2015), will overcome these shortcomings; however, this has so far not yet been successfully applied to seeds.

In conclusion, we show that dry seed mRNAs are associated with ribosome complexes, which likely ensures their survival during seed dry storage. In Figure 4C, we show a model presenting the fate of seed-stored mRNA. The association with monosomes likely safeguards the mRNAs needed during early seed imbibition in a state ready for translation (Bai et al., 2017). Sequence features such as secondary structure and specific motifs in the mRNAs might play a role in selecting mRNAs either for storage or translation; however, more experimental evidence is needed to fully describe the mechanism. Our data confirm that mRNAs are programmed for storage during the final stage of seed development for translation during imbibition, which can occur years later. This truly remarkable length of time separating the regulatory decision and execution is in contrast with the usually very short turnover of mRNA molecules in active cells.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds of the *Arabidopsis* (*Arabidopsis thaliana*) accession Columbia-0 were used for all assays described. The time points for sampling seeds and seedlings were according to Bai et al. (2017). Completely nondormant seeds were sampled in their dry state (seed) and after 72 h in germination promoting conditions (moisture, continuous light [$140 \mu\text{mol m}^{-2}\text{s}^{-1}$] at 22°C; seedling).

For ribosome analyses, seeds and green seedlings were harvested and frozen in liquid nitrogen followed by freeze-drying. The freeze-dried material was stored at -80°C until further analyses.

Isolation of Total RNA and Polysomal RNA and Polysome Analysis

For the isolation of mRNA bound to ribosomes, ~ 400 mg of freeze-dried tissue was extracted with 8 ml of polysome extraction buffer (0.25 M Suc, 400 mM Tris [pH 9.0], 200 mM KCl, 35 mM MgCl_2 , 5 mM EGTA, 50 $\mu\text{g}/\text{mL}$ cycloheximide, 50 $\mu\text{g}/\text{mL}$ chloramphenicol; Bai et al., 2017). The extracts were loaded on top of a Suc cushion (1.75 M Suc in polysome extraction buffer) and centrifuged (18 h, 90,000 g) using a Beckman Ti70 rotor (Beckman Coulter). The resulting pellet was resuspended in wash buffer (200 mM Tris [pH 9.0], 200 mM KCl, 0.025 M EGTA, 35 mM MgCl_2 , 5 mM dithiothreitol, 50 $\mu\text{g}/\text{mL}$ cycloheximide, and 50 $\mu\text{g}/\text{mL}$ chloramphenicol) and loaded on a 20–60% (w/v) linear Suc gradient, centrifuged at 190,000 g for 1.5 h, at 4°C using Beckman SW55 rotor (Beckman Coulter). After ultracentrifugation, the gradients were fractionated into 20 fractions using a Teledyne Isco Density Gradient Fractionation System (Teledyne Isco) with online spectrophotometric detection of (254 nm). The fractions were pooled into samples containing monosome and polysome complexes. Ribosome abundance was measured by the area under the curve and was calculated after subtracting the absorbance obtained from blank gradient and normalizing to total area under the curve to account for possible uneven loading of the gradients. The profile was obtained by loading ribosomes extracted from equal amounts of freeze-dried material and by loading equal amounts of ribosomes (evaluated by optical density 254) to the Suc gradient, respectively.

Quantification of mRNA

The quantification of mRNA species from the ribosome fraction and the unprecipitated supernatant is based on a total RNA isolation using TriPure Isolation Reagent (Roche). Further purification was performed RNeasy Mini spin columns (Qiagen), and the RNA was dissolved in RNase-free H_2O for further analyses. To evaluate the mRNA content in both free and ribosome-associated fractions, total RNA from both fractions was isolated and the mRNA species were purified based on equal total RNA input by Dynabeads Oligo(dT) 25. The amount of mRNA was quantified by Qubit RNA HS Assay Kit (ThermoFisher). The mRNA distribution in each fraction was calculated by the ratio of mRNA either from the ribosome pellet or the free fraction from the supernatant to the total mRNA in the seeds. Student's *t* tests were performed to compare the difference in mRNA distribution of both fractions in both seeds and seedlings.

Microarray Data Analysis

Affymetrix Arabidopsis Gene 1.1 ST Arrays (Affymetrix) were hybridized using the GeneChip 3' IVT Express kit (cat. # 901229) according to instructions from the manufacturer. Hybridization data were analyzed, and gene-specific signal intensities were computed using the R statistical programming environment (www.R-project.com), the BioConductor package Affy (Gautier et al., 2004), and the Brainarray cdf file ver. 17.1.0 (<http://brainarray.mbni.med.umich.edu/>). DNA microarray data are available in the Gene Expression Omnibus repository (<http://www.ncbi.nlm.nih.gov/geo/>) under accession number GSE76905. Following Robust Multichip Average (RMA) normalization (Irizarry et al., 2003), intensity signals that never exceeded our noise threshold ($\log\text{Exprs} < 4$ in all samples) were removed. The LIMMA package was used to obtain differential gene expression data with adjusted *P*-value < 0.05 , $\text{HDR} < 0.05$, $\text{Log}(\text{FC}) > 1$ as cutoff (Smyth, 2005; Diboun et al., 2006). Correlation between RMA-normalized biological replicates averaged at 0.97 (Pearson's correlation) and ranged between 0.96 and 0.99 (Supplemental Fig. S2). Relative RNA levels were validated with RT-qPCR experiments with four spikes as internal standard for the normalization (Vandesompele et al., 2002). In general, 2 μL of the mixed four Eukaryotic Poly-A RNA controls (cat. # 1205031) were spiked into each of the gradient fractions according to the manufacturer protocol (Affymetrix), which is used for data normalization. DNA sequences and efficiencies of primer pairs used for RT-qPCR experiments and comparison of relative mRNA levels determined in GeneChip and RT-qPCR experiments are given in Supplemental Table S3.

Sequence Feature Analysis

The four different gene sets (Seed Monosome, Seed Polysome, Seedling Monosome, and Seedling Polysome) were divided into different length bins

(<3000 nt, 3000–5000 nt, >5000 nt) to compare our data with a similar dataset from yeast (Heyer and Moore, 2016). These groups were compared with each other and with the microarray background (all mRNAs expressed in the experiment) for several sequence features using custom scripts. The distributions of sequences length, GC content, and nt content were evaluated separately for CDS, 5'UTR, 3'UTR, and full transcript. CDSs were also analyzed for GC3 content, after removing sequences missing the start codon and/or containing premature stop codons; CDSs shorter than 100 codons were further removed for the codon bias analysis, measured using the effective Nc index (Sun et al., 2013). The same analyses were performed separately for the CDS of protein-coding genes having both or no annotated UTR (UTRs called present when having length >1 nt). *P*-values were calculated by ranked sum Wilcoxon test (length and codon bias) or *t* test (GC%, GC3%, and nt content).

RNA Structure and Stability Analysis

Experimentally determined structure scores per nucleotide, as provided by (Li et al., 2012), were used to calculate average structure scores of the genes with significantly increased and decreased ribosomal association for each gene set (seeds or seedlings, and monosome or polysome). Relative scaling was achieved by averaging the structure scores per region (5'UTR, CDS, and 3'UTR) in 100 bins in comparison with the background (all mRNAs expressed in the experiment). SES and Student's *t* tests were performed using the Python SciPy module (<http://www.scipy.org/>). Transcript stability was evaluated by retrieving data from Narsai et al. (2007). Student's *t* tests for the transcript stability were performed using R (<https://www.r-project.org/>).

Motif Analysis

DNA motif analyses were performed using the MEME suite (Bailey et al., 2009), for full transcript, 5'UTR, CDS, and 3'UTR sequences of each gene set (DS or GS, and monosome or polysome), extracted from the The Arabidopsis Information Resource (TAIR)10 genome compilation (<http://www.arabidopsis.org/>). The minimum and maximum motif width was set to 6 and 10, respectively. If a gene had multiple isoforms, only the TAIR10 representative splice form was used. Background dinucleotide frequencies were provided separately for each sequence type. The resulting motifs with an E-value <1E-5 were used by FIMO (Bailey et al., 2009) to retrieve all locations of the motif of all the transcripts in the background (all transcripts present on the microarray). This information was used to calculate the average number of times a motif is present at a specific position (relative number of motifs) for five distinct regions for each gene set and the background. These regions were at the 5' end of the transcript and downstream, upstream of the start codon, in the middle of the coding DNA sequence (CDS), downstream of the stop codon, and from the 3' end upstream. Each region is 50 nucleotides in length. These results were used to create graphs and calculated enrichment or depletion of the motif at certain positions for each gene set including *P*-values and fold changes (Fig. 3B; Supplemental Fig. S9B) using a custom script (python) and the R software package (<http://www.r-project.org/>). All gene sets and positions with a *P*-value <1E-5 in either monosome- or polysome-specific transcripts are shown.

Ribosomal Protein Extraction, Tryptic Digestion, and Peptide Purification

Ribosome fractionation was performed as described above. The fractions corresponding to monosome and polysome regions were separately pooled, and 1.1 mL of methanol was added to and 0.6 mL of each pooled ribosome extract and mixed thoroughly. After 0.3 mL of chloroform was added, vortexed, and centrifuged for 15 min at 16,000 g, the supernatant was carefully removed with a capillary pipette. The air-dried protein pellet was dissolved in 100 μ L of 8 M urea, and an aliquot was taken for protein estimation using the Qubit protein HS Assay Kit (ThermoFisher). Between 4 and 15 μ g of protein dissolved in 100 μ L of 8 M urea was used for digestion after reduction (5 mM dithiothreitol; 30 min at 37°C) and alkylation (15 mM iodoacetamide; 30 min at RT in the dark) of the cysteines. After 75 μ L of 0.1 M ammonium bicarbonate buffer (pH8.3) was added, 25 μ L of Trypsin/Lys-C Mix (0.02 μ g/ μ L, Promega) was added for digestion (3 h at 37°C). After dilution with 200 μ L of 0.1 M ammonium bicarbonate, 4 μ L of modified trypsin (0.05 μ g/ μ L, Promega) was added for overnight incubation at 37°C. Digestion was terminated by adding 0.1% (v/v) trifluoroacetic acid. The tryptic digests were cleaned by reverse phase solid phase extraction on an Oasis HLB Elution Plate (Waters Corporation). Peptides

were eluted with 0.1 mL of 50% (v/v) acetonitrile (ACN) and 0.1% (v/v) trifluoroacetic acid, and dried by vacuum centrifugation. For 2D-LC-MS analysis, the peptides were dissolved in 20 μ L of 0.1 M ammonium formate (pH 10) and 5 μ L of the peptides were injected into the machine.

Chromatography and Mass Spectrometry

For high resolution separation of the ribosomal protein digests, a nano-Acquity 2-D UPLC system (Waters Corporation) was used employing orthogonal reverse phase separation at high and low pH, respectively. With this 2-D setup, the pool of peptides was eluted from the first dimension XBridge C18 trap column (in 20 mM ammonium formate pH 10) in two steps of 20 and 65% (v/v) ACN. For the second dimension, we used a BEH C18 column (75 μ m \times 25 cm, Waters Corporation) eluting with a 100-min linear gradient from 3% to 40% (v/v) ACN (in 0.1% [v/v] formic acid) at 200 nL/min. The eluting peptides were on-line injected into a Q-Exactive Plus (Thermo Scientific) mass spectrometer using a nano-electrospray source. Ionization (2.4 kV) was performed using a stainless-steel emitter and a heated capillary temperature of 250°C. Full MS scans were acquired over the *m/z* range 400 to 1500 with a mass resolution of 70,000 (at *m/z* 200). Full scan target was set 3×10^6 with a maximum fill time of 50 ms. The 10 most intense peaks with charge state 2–4 were fragmented in the HCD collision cell with a normalized collision energy of 28%. The mass range was set to 140–2000, and a mass resolution of 17500 (at *m/z* 200). The target value for fragment scans was set at 5×10^4 , the intensity threshold was kept at 1×10^4 , and the maximum allowed accumulation times were 100 ms. Peptide match was set to preferred, isolation window at 1.6 *m/z*, and isotope exclusion was on. The dynamic exclusion was set to 30 s.

Database Search for Protein Identification and Quantitative Analysis

The raw QEX LC-MS/MS data were processed with the work flow described for plant proteomics using the Maxquant (Cox and Mann, 2008; Smaczniak et al., 2012), and the resulting mass file containing all the fragment information was matched against the in-house TAIR protein sequence database based on The Arabidopsis Information Resource (TAIR10, www.Arabidopsis.org). The proteinGroups.txt file generated from Maxquant was used for quantitative analysis. Intensity determination and normalization was performed by algorithm MaxLFQ (Cox et al., 2014). The label-free quantification (LFQ) value generated was used for further statistics analysis by package Perseus (Tyanova et al., 2016). A protein was identified as present if Log2 transformed LFQ value is above background for at least the three biological replicates in either monosome or polysome fractions. An imputation was used to simulate the missing values based on the normal distribution with simulation width of 0.3 and shift of 1.8. Student *t* test is used to identify proteins enriched in either monosome fractions or polysome fractions with FDR adjusted *P*-value <0.05 and fold change >2. For ribosomal protein analysis, the reannotated ribosomal proteins from (Hummel et al., 2015) were extracted from the raw data in both proteinGroups.txt file and peptides.txt to retrieve protein and peptide information of all identified ribosomal proteins. The unique peptides were selected for each ribosomal protein paralogs when they are matched to only single ribosome in all samples of seed monosome and polysome fractions. To compare the composition of ribosome in monosome and polysome fractions, the raw LFQ value was normalized by the total sum of LFQ of all unique ribosomal peptides in either monosome or polysome fractions, which removes bias of unequal distribution of ribosomes in monosome and polysome fractions.

Gene and Protein Function Classification and Overrepresentation Analysis

DAVID Bioinformatics Resources 6.7 were used for gene functional annotation and GO enrichment analysis (Huang et al., 2009). Panther classification system was used for ribosome protein classification and statistical overrepresentation test (Mi et al., 2013). Panther protein class was used as reference list, which contains 26,684 proteins in the database. Bonferroni corrected protein list was generated with *P*-value <0.05.

Accession Numbers

All the identified genes and proteins are provided in the Supplemental Tables S2 and S4, respectively.

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Gene 1.1 ST Genechip quality assessment and reproducibility.

Supplemental Figure S2. Evaluation of sample reproducibility.

Supplemental Figure S3. Sequence length characteristics of monosome (M) and polysome (P) associated transcripts in dry seeds.

Supplemental Figure S4. GC content, codon bias and transcript abundance of monosome specific and polysome specifically associated transcripts in dry seeds.

Supplemental Figure S5. Turnover and intron number of monosome (A) and polysome (P) specifically associated transcripts in dry seeds.

Supplemental Figure S6. Monosome loading and sequence length of monosome (M) or polysome (P) specifically associated transcripts in seedlings.

Supplemental Figure S7. GC content, codon bias and transcript abundance of monosome (M) and polysome (P) specifically associated transcripts in dry seeds.

Supplemental Figure S8. Turnover and intron number of monosome and polysome specifically associated transcripts in seedlings.

Supplemental Figure S9. Secondary structure and motifs identified in transcripts specifically associated with monosome and polysome fractions in seedlings.

Supplemental Figure S10. Validation of the secondary structure analyses for the monosome specifically associated mRNAs.

Supplemental Table S1. RMA-normalized and filtered dataset used for statistical analysis.

Supplemental Table S2. Monosome and polysome specifically associated transcripts in both seed and seedling.

Supplemental Table S3. qPCR confirmation of the monosome/polysome specifically associated transcripts.

Supplemental Table S4. Ribosome proteome identified in seed. In total, 1398 proteins are identified in either monosome or polysome fractions.

Supplemental Table S5. Protein functional groups of monosome and polysome specifically associated proteins in seed.

Supplemental Table S6. Total ribosomal protein identified in seed ribosome proteome.

Supplemental Table S7. Total ribosomal protein unique peptides identified in seed ribosome proteome.

Supplemental Table S8. GO enrichment analysis of monosome/polysome specifically associated transcripts that are up/down translationally regulated at early hydration in seed.

Supplemental Table S9. GO enrichment analysis of monosome/polysome specifically associated transcripts in seed.

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