Expression changes of ribosomal proteins in phosphate- and iron-deficient Arabidopsis roots predict stress-specific alterations in ribosome composition

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Abstract

**Background:** Ribosomes are essential ribonucleoprotein complexes that are engaged in translation and thus indispensable for growth. *Arabidopsis thaliana* ribosomes are composed of 80 distinct ribosomal proteins (RPs), each of which is encoded by two to seven highly similar paralogous genes. Little information is available on how RP genes respond to a shortage of essential mineral nutrients such as phosphate (Pi) or iron (Fe). In the present study, the expression of RP genes and the differential accumulation of RPs upon Pi or Fe deficiency in Arabidopsis roots were comprehensively analyzed.

**Results:** Comparison of 3,106 Pi-responsive genes with 3,296 Fe-responsive genes revealed an overlap of 579 genes that were differentially expressed under both conditions in Arabidopsis roots. Gene ontology (GO) analysis revealed that these 579 genes were mainly associated with abiotic stress responses. Among the 247 RP genes retrieved from the TAIR10 release of the Arabidopsis genome (98 small subunit RP genes, 143 large subunit RP genes and six ribosome-related genes), seven RP genes were not detected in Arabidopsis roots by RNA sequencing under control conditions. Transcripts from 20 and 100 RP genes showed low and medium abundance, respectively; 120 RP genes were highly expressed in Arabidopsis roots. As anticipated, gene ontology (GO) analysis indicated that most RP genes were related to translation and ribosome assembly, but some of the highly expressed RP genes were also involved in the responses to cold, UV-B, and salt stress. Only three RP genes derived from three ‘sets’ of paralogous genes were differentially expressed between Pi-sufficient and Pi-deficient roots, all of which were induced by Pi starvation. In Fe-deficient plants, 81 RP genes from 51 ‘sets’ of paralogous RP genes were significantly down-regulated in response to Fe deficiency. The biological processes ‘translation’ (GO: 0006412), ‘ribosome biogenesis’ (GO: 0042254), and ‘response to salt’ (GO: 0009651), cold (GO: 0009409), and UV-B stresses (GO: 0071493) were enriched in this subset of RP genes. At the protein level, 21 and two RPs accumulated differentially under Pi- and Fe-deficient conditions, respectively. Neither the differentially expressed RP genes nor the differentially expressed RPs showed any overlap between the two growth types.

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Conclusions: In the present study three and 81 differentially expressed RP genes were identified under Pi and Fe deficiency, respectively. At protein level, 21 and two RP proteins were differentially accumulated under Pi- and Fe-deficient conditions. Our study shows that the expression of paralogous genes encoding RPs was regulated in a stress-specific manner in Arabidopsis roots, presumably resulting in an altered composition of ribosomes and biased translation. These findings may aid in uncovering an unexplored mechanism by which plants adapt to changing environmental conditions.

Keywords: Iron deficiency, Phosphate deficiency, Proteomics, Ribosomal proteins, RNA-seq, Translation

Background
Ribosomes are large ribonucleoprotein complexes which synthesize the vast majority of cellular peptides. The structure of ribosomes is essentially the same in prokaryotes and eukaryotes in that they are composed of ribosomal RNAs (rRNAs) and ribosomal proteins (RPs), organized in a large and a small subunit. However, ribosomes of eukaryotes exhibit greater structural diversity, reflecting the higher complexity of the molecular mechanism underlying translation in the latter group [1,2]. In Arabidopsis thaliana, cytoplasmic ribosomes are composed of four distinct rRNAs (the large subunit contains 26S, 5S and 5.8S rRNA, the small subunit contains 18S rRNA) and 80 distinct ribosomal proteins (32 proteins in the small subunit and 48 proteins in the large subunit) [3,4]. The 80 RPs are encoded by a total of 249 genes; thus, several paralogous genes encode the same RP [1,4]. These paralogous genes could be redundant or, as indicated for certain RP genes, may be involved in specific plant processes or developmental stages [5-10]. For example, among the four paralogous genes encoding RPL11, one gene was highly expressed in cotyledons, whereas the other three genes were preferentially expressed in roots, developing anthers and pollen [11]. In addition, it has been reported that a mutation in RPL23aA (one out of two paralogous gene encoding RPL23a) lead to impaired growth and developmental abnormalities, suggesting that RPL23aA plays an essential role in fitness traits. By contrast, knockdown of the closely related gene family member RPL23ab had little effect on the phenotype [7]. Similarly, genes encoding RPS15a were differentially expressed in Arabidopsis, with one paralogous gene being completely transcriptionally quiescent, while the other three were highly expressed in mitotically active regions (e.g. flowers and buds) [12].

Ribosomal proteins are essential for protein synthesis and, consequently, play an important role in metabolism, cell division, and growth. In addition to their housekeeping functions, the phenotypes resulting from mutations in several different RP genes provide strong evidence that RPs participate as regulatory components in developmental processes [13]. Generally, RP mutants share developmental abnormalities such as reduced shoot growth, reduced cell proliferation and increased nuclear ploidy in leaf cells [13-15]. For example, a semi-dominant mutation in RPL27aC affected multiple aspects of plant shoot development, including leaf patterning, inflorescence and floral meristem function, as well as seed set [16]. Silencing of RPS10 disturbed the ratio between the small and large subunits of mitoribosomes, causing an excess of the latter [17]. Introducing RPS6 antisense and RPL23aA RNAi constructs resulted in an altered number of cotyledons [7,18]. Also, a dominant missense mutation in RPL10A suppressed stem-elongation in comparison with the wild type [19]. Less severe phenotypes were reported for mutations in other RP genes. POINTED FIRST LEAF (PFL) encodes the small subunit RPS18 [20]. pfl mutants showed changes in the shape of early vegetative leaves from the spatulate wild-type shape to a pointed, narrow shape. Mutations in the PFL2 (RPS13) gene caused a delay in the transition to flowering and the production of more vegetative leaves than in the wild type [21]. In addition to effects on plant development and growth, RPs also took part in the response to stress. Under UV-B stress, RPL10 genes were differentially regulated in a dosage- and time-dependent manner; while RPL10C was induced and RPL10B was down-regulated at high UV-B intensity, RPL10A was not responsive to UV-B [22]. Another study showed that specific RPs changed in abundance in response to sucrose feeding, implying that different RPs are incorporated into ribosomes depending on the growth condition [23]. Transcripts of RPS15aF increased following treatment with cytokinin 6-benzylaminopurine (BAP) and auxin indole acetic acid (IAA), while abscisic acid (ABA) treatment decreased transcript abundance. In addition, transcripts of RPS15aA, RPS15aD and RPS15aF showed increased abundance upon temperature and mechanical stress [12].

Phosphate (Pi) is an essential macronutrient for plants. In addition to its structural role in nucleic acids and cell membranes, Pi has important functions in lipid and energy metabolism. Limited bioavailability of Pi often restricts growth in natural and agricultural ecosystems. Due to intense use of P fertilizers in agriculture, Pi...
resources have become limited. Therefore, understanding how plants adapt to low Pi availability is of critical importance to generate Pi-efficient plants. As a coping mechanism to limited Pi supply, plants undergo dramatic changes in metabolism, physiology, hormonal balance and gene expression [24-26]. Similarly, iron (Fe) is essential for the survival and proliferation of virtually all organisms. Iron is required for many vital enzymatic reactions such as DNA replication, lipid metabolism, and nitrogen fixation. In plants, Fe deficiency is a common nutritional disorder worldwide, causing low yield and quality of crop plants. To acquire sufficient Fe while avoiding toxicity, plants tightly control uptake, utilization, and storage in response to environmental availability [27].

At present, most studies on plant RPs focus on the developmental effects of RP mutants. We recently showed that Fe deficiency alters the expression of several RPs, presumably resulting in biased translation [28]. In the present study, changes in global expression pattern of RP genes in Arabidopsis roots upon Pi and Fe deficiency were compared at the transcriptomic and proteomic level in order to gain insights into the regulation of the response to Pi or Fe deficiency. It is shown that the expression of RPs followed stress-specific pattern, revealing a novel layer of regulation that may be critical for the fitness of plants under nutrient shortage.

Results and discussion
Comprehensive expression analysis of ribosomal protein genes in Arabidopsis roots
The RNA-seq technology has proven to provide precise gene expression information [29] and accurate discrimination of differentially expressed genes under various conditions. Using this technology, we previously analyzed global gene expression changes upon Pi-deficiency and Fe-deficiency in Arabidopsis roots [30,31]. A total of 24,591 genes were detected in roots from Pi-deficient and Pi-sufficient plants by alignment to the TAIR10 annotation of the Arabidopsis genome (Figure 1). A set of 3,106 genes (13%) was differentially expressed under Pi deficiency (t-test, P value <0.05, of which 1,497 genes were up-regulated and 1,609 genes were down-regulated). Using the same criteria, a total of 3,296 genes (14%, 1,462 induced genes and 1,834 depressed genes) were found to be differentially expressed between Fe-sufficient and Fe-deficient conditions among the 24,319 detected genes. Thus, a comparable number of genes were affected in both growth types. A subset of 579 genes was differentially expressed under both conditions.

Ribosomal proteins play critical roles in ribosome biogenesis, translation and post-translational modifications of proteins. A complete sequence data set for the 80 known RP families was compiled from the TAIR10 release of the Arabidopsis genome (http://www.arabidopsis.org/browse/genefamily/athr.jsp) [4,32]. This data set (n = 256, Additional file 1) comprises all Arabidopsis paralogous genes associated with any specific RP family and other ribosome-related genes including eIF6 (eukaryotic Translation Initiation Factor 6), RACK1 (Receptor of Activated C Kinase) [33] and FER3 (FERRITIN 3) [34], which was shown to be tightly associated with ribosomes [5]. We defined the expression level of all RP genes in this data set according to an earlier study [35]. If the number of unique reads was zero in all three biological repeats under normal (Pi-/Fe-sufficient) condition, the gene was defined as not expressed; low abundance of a transcript was defined by a number of unique reads <10 in either of the three biological repeats; medium abundance was defined for genes with 11 to 2000 reads, and genes with more than 2000 reads in either of the three repeats were defined as highly expressed. On the basis of these criteria, in Arabidopsis roots subjected to Pi deficiency seven RP genes were not detected, transcripts of 20 RP genes were lowly abundant, 100 RP genes showed medium transcript levels, and 120 RP genes were highly expressed (Figure 2A and Additional file 2). The average read number of RP genes was 2,803; while on average 456 reads were associated with non-RP transcripts, indicating that RP paralogs were highly expressed in Arabidopsis roots. Previous data suggested that RP genes are retained in the Arabidopsis genome at higher levels than other types of gene [36], suggesting a high biological importance of this plant-specific diversity of RP genes. In growing yeast cells, 2000 ribosomes were produced per minute, and transcription of ribosomal RNA and RP genes account for 60% of total transcription [37]. The synthesis of RPs, ribosome assembly factors, and extra-ribosomal components involved in translation must simultaneously satisfy the plant's demand for metabolism and growth, comprising an important component of the total cellular translational activity.
Figure 2 Expression level of RP genes and proteins in Arabidopsis roots. If the unique read number was zero in all three biological repeats under normal (Pi-/Fe-sufficient) conditions, the gene was defined as not expressed; low abundance of a transcript was defined by a unique read number <10 in either of three biological repeats; medium abundance was defined as read number between 11 and 2000, and highly expressed genes had more than 2000 reads in either of three biological repeats. (A) Read counts and gene numbers, (B) Percentage of the small subunit of ribosomal proteins identified in Arabidopsis roots, (C) Percentage of the large subunit of ribosomal proteins (D) Percentage of the ribosomal-related proteins.
In many cases, different RP paralogs from the same RP families displayed differential expression. For example, among three paralogs of the RPL18 family, RPL18B and RPL18C were highly expressed with more than 5,000 reads, while the third member of this family, RPL18A, was not expressed. By analyzing public transcriptomic data (Genevestigator, https://www.genevestigator.com/gv/plant.jsp) [38], we found that expression of RPL18A is particularly low among all tissues and treatments, whereas RPL18B and RPL18C are highly expressed in some tissues. This suggests that RPL18A may be not functional or only of minor importance, although retained in the genome during evolution. Pronounced differences were also observed for the expression of genes encoding the proteins of the large subunits L4, L22, L27a, L30, L37a, and L41. In a previous report, RPS15aA, RPS15aD and RPS15aF, but not RPS15ac transcripts were detected in all mature tissues by semi-quantitative RT-PCR [12]. Taken together, the differential expression of genes encoding RPs within specific ribosomal gene families indicates extensive ribosome heterogeneity at the intercellular level [6].

Within the 13 RP gene families with high read numbers (>2000) in Arabidopsis roots were six small (S3, S3a, S5, S6, S13, and S24) and seven large (L6, L7a, L10a, L15, L17, L24, and L38) ribosomal subunit proteins (Additional file 2). The highest expression level was observed for S3a, which was detected with an average read number of 6,917 for each paralog. High steady state levels of some RP gene transcripts may be indicative of fundamental roles of the encoded proteins in plant growth and development.

At the protein level, for 241 RP genes and 6 ribosome-related genes a total of 125 cognate proteins, accounting for nearly 50% of the detected genes, were identified in a proteomic approach ([30] and Additional file 3). This accounts for 75% of the large subunit proteins, 87.5% of the small subunit proteins, and 100% of the ribosome-related proteins. These results suggest that some RP families are necessary for translation in Arabidopsis roots, but not all of the paralogous proteins within one RP family take part in this process. Our data further demonstrate that RPs were more likely to be detected among the highly expressed genes than in genes with transcripts of medium or low abundance (Figure 2B, 2C and 2D). Notably, 41 highly expressed RP genes had no cognate peptides, supporting the observation that the gene expression level is not always correlated with protein abundance [30,35]. Many RP paralogs might have been detected, but without unique matches due to their high sequence identity. For example, for three paralogous proteins of the RPL6 family, RPL6A (AT1G18540), RPL6B (AT1G74060) and RPL6C (AT1G74050), 21 matching peptides have been detected, but only for RPL6A seven unique peptides were found, defining RPL6B and RPL6C as not present at the protein level although their corresponding transcript are high.

Gene Ontology (GO) enrichment analysis revealed that the products of most of the RP genes were localized in the cytosolic small and large ribosomal subunits, especially proteins encoded by medium and highly expressed genes (Figure 3A, P < 0.01). In addition, some biological processes such as ‘cellular response to UV-B’ (GO: 0071493), ‘response to cold’ (GO: 0009409), and ‘response to salt stress’ (GO: 0009651) were overrepresented among the highly expressed genes (Figure 3B), indicating that RPs may also participate in the response to abiotic stresses. In addition, the GO categories ‘seed germination’ (GO: 0009845), ‘embryo development ending in seed dormancy’ (GO: 0009793), and ‘leaf morphogenesis’ (GO: 0009965) were overrepresented in this group of RP genes (Additional file 4). In previous studies, mutations in some RP genes (i.e. RPS5B, RPS56B, RPS11A, RPL3A, RPL8A, RPL19A, RPL23A, and RPL40B) caused an embryo-lethal phenotype, [13,39,40]; thus, shortage in specific RPs could lead to severe growth defects. Mutations in the RP genes S13 and S18 were reported to cause abnormal leaf development [20,21]. Changes in leaf morphology in RP gene mutants might be caused by a diminished capacity to maintain protein synthesis at a level sufficient for proliferative cell divisions in the vasculature and palisade mesophyll, and for cell division progression at the leaf margin [13].

Expression of RP genes in Pi-deficient Arabidopsis roots

Out of the 241 RP genes and six ribosomal-related genes annotated in TAIR10, only three were differentially expressed between Pi-sufficient and Pi-deficient Arabidopsis roots (t-test, P < 0.05; Table 1), all of which were up-regulated. Among the three genes, induction of At1g74270 (RPL35aC) was more pronounced when compared with the other two genes, AT3G04230 (RPS16B) and AT3G02190 (RPL39B). In rice flowers, RPL35a was highly expressed under high temperature after the flowering stage [41], suggesting that this RP family is not only involved in Pi deficiency but may also play a role in heat stress response. In a previous report, transcript levels of RPS16, RPL30 and RPL3A from Lupine cotyledons were found to be increased during cytokinin treatment relative to a 0 h water control [42]. Expression of RPS16 was decreased after 5 h of incubation with ABA, indicating that RPS16 may have different functions in abiotic stresses.

The rapid accumulation of genome-wide gene expression data allows the creation of co-expression networks by examining the pair-wise relationship of genes over a large number of experimental conditions. In the co-expression network, a node is represented by a gene,
and an edge is drawn between gene A and B if the correlation coefficient between these two genes is above a defined threshold [43]. Genes with similar expression pattern may be involved in the same biological process and potential functions of the unknown genes in a co-expression network can be predicted. [44,45]. In an attempt to identify Pi-responsive non-RP genes that are co-regulated with RP genes, we analyzed the correlation of the expression of the 3,106 differentially expressed genes including three RP genes. The results show that only one RP genes (AT3G04230) interacted with the 2-oxoglutarate (2OG) and Fe (II)-dependent oxygenase superfamily protein AT3G13610 with a Pearson correlation coefficient (CF) cutoff of 0.7. With this CF threshold decreased to 0.5, all three differentially expressed RP genes and 262 other differentially expressed genes including some transcription factors and protein kinases could form a larger co-expression network (Additional file 5); and these genes were involved in diverse biological processes (Additional file 5).

At the protein level, 21 RPs accumulated differentially upon Pi deficiency (Table 2). Among them, only RPL12B (AT3G53430) was up regulated, all other proteins showed decreased abundance. Interestingly, among the three paralogous proteins in the RPL12 family, only RPL12B was induced, while the abundance of the other two RPL12 members was unchanged under Pi-deficient conditions. This suggests that RPL12B may play an important role in the Pi-stress response. To get a better understanding of the function of differentially expressed RPs, a protein-protein interaction network was constructed using the STRING database (http://string-db.org) [46] The network is presented in the ‘evidence’ view, whereby lines connecting proteins represent the type of evidence (neighborhood, gene fusion, co-occurrence, co-expression, experiments, databases, text mining) used in predicting the association. All 21 differentially expressed RP proteins were clustered

Table 1 Differentially expressed RP genes in roots of Pi-deficient plants

| Gene ID   | Protein name | Gene name | Type          | Mean ± SD (−Pi/+Pi; P < 0.05) |
|-----------|--------------|-----------|---------------|-------------------------------|
| AT3G04230 | S16          | RPS16B    | Small subunit | 1.210 ± 0.085                 |
| AT3G04270 | L35a         | RPL35aC   | Large subunit | 1.425 ± 0.206                 |
| AT3G02190 | L39          | RPL39B    | Large subunit | 1.224 ± 0.086                 |

Figure 3 Gene ontology (GO) enrichment analysis of the four types of RP genes in Arabidopsis roots. (A) Subcellular location of the four types of RP genes. (B) Biological process of the four types of RP genes.
in a large protein-protein interaction module (Additional file 6).

Alternative splicing (AS) is a mechanism that increases transcriptome and proteome complexity and controls developmental programs and responses to the environment in higher eukaryotes [47,48]. Previously, genome-wide differential AS features induced by Pi deficiency have been analyzed in Arabidopsis roots [31]. Further analysis revealed that a subset of 14 RP genes produced transcripts with retained introns; all of these intron retention events were induced by Pi shortage (i.e. more transcripts with retained introns were produced under Pi-deficient conditions; Additional file 7). Three out of the 14 encoded RPs were decreased in abundance under Pi deficiency. These results suggest that post-transcriptional regulatory processes such as alternative splicing may have affected the efficiency of translation [49].

Expression of ribosomal protein genes in Fe-deficiency Arabidopsis roots
Using the criteria listed above, a total of 81 RP genes were differentially expressed in Fe-deficient Arabidopsis roots. In contrast to the up-regulated pattern of differentially expressed RP genes in roots of Pi-deficient plants, expression of all the 81 RP genes was decreased upon Fe deficiency. The RP gene with lowest relative expression level was the ribosome-associated protein gene FERRITIN3 (FER3; AT3G56090), which was found in small polysome fractions isolated form the leaves of Arabidopsis plants in the dark [34]. FER3 was reported to be associated with the chloroplast and suggested to mediate coordination between the translation activity of cytosolic ribosomes and the function of chloroplasts in response to iron-based signals [50]. Thus, decreased expression levels of FER3 under Fe deficiency may be associated with the regulation of cellular Fe homeostasis.

Gene ontology analysis revealed that the 81 differentially expressed RP genes were chiefly involved in translation and ribosome biogenesis. In addition, the biological processes ‘response to salt stress,’ ‘cellular response to UV-B,’ and ‘leaf morphogenesis’ were also enriched (Figure 4; Additional file 8). The 81 differentially expressed genes were derived from 23 small and 26 large ribosomal subunit RPs (Table 3), in which the smallest ribosomal subunit RPs (Table 3), in which the transcript abundance of all paralogous genes from five RP families (L10a, L7a, S14, S3 and S5), was decreased. In the RPS15a family, three of the six paralogous genes (RPS15aA, RPS15aB, and RPS15aD) were down-regulated under Fe deficiency. These three genes showed increased transcript abundance following treatment with BAP, RPS15aF was also induced by IAA treatment. A similar trend of expression in paralogous genes with RPS15a family was observed under heat and cold stress [12]. It might thus be assumed that RPS15a is an important regulator involved in multiple abiotic and nutrient stresses.

Co-expression analysis of the 81 differentially expressed RP genes under Fe deficiency yielded a network consisting of 68 nodes and 1,827 edges (Additional file 9). This network could be further divided into one larger and one smaller clusters, the larger of which contained much more nodes (66/68) and edges, and every node (ribosomal protein gene) was correlated with other RP genes (27 edges per node). Similarly, with the aim of identifying other Fe-responsive non-RP genes that interact with RP genes, we put the 3,296 differentially expressed genes including 81 RP genes together to do the co-expression analysis. Results showed that a total of 77 RP genes and 221 Fe-responsive non-RP genes comprise one bigger and two smaller clusters with Pearson correlation coefficient cutoff of 0.7 (Additional file 10).

Proteomic profile analysis showed that only two RPs were differentially expressed between Fe-deficient and Fe-sufficient Arabidopsis roots [49]. The two proteins were from the RPL22 family and showed decreased abundance, although the transcriptional expression of these two genes remained unchanged (Table 4). Analysis of AS revealed three RP genes with differential alternative donor/acceptor splices sites and six RP genes with

### Table 2 Differentially expressed ribosomal proteins in roots of Pi-deficient plants

| Protein ID | Protein name | Gene name | Type   | Fold change |
|------------|--------------|-----------|--------|-------------|
| AT5G20290 | S8           | RPS8A     | Small subunit | 0.738 ± 0.114 |
| AT3G64040 | S15a         | RPS15aD   | Small subunit | 0.822 ± 0.039 |
| AT2G09990 | S16          | RPS16A    | Small subunit | 0.810 ± 0.094 |
| AT5G02960 | S23          | RPS23B    | Small subunit | 0.561 ± 0.166 |
| AT4G9200  | S25          | RPS25E    | Small subunit | 0.689 ± 0.081 |
| AT3G56340 | S26          | RPS26C    | Small subunit | 0.673 ± 0.186 |
| AT1G18540 | L6           | RPL6A     | Large subunit | 0.831 ± 0.078 |
| AT3G62870 | L7a          | RPL7aB    | Large subunit | 0.825 ± 0.032 |
| AT2G18020 | L8           | RPL8A     | Large subunit | 0.777 ± 0.227 |
| AT1G08360 | L10a         | RPL10aA   | Large subunit | 0.831 ± 0.068 |
| AT3G53430 | L12          | RPL12B    | Large subunit | 1.385 ± 0.080 |
| AT3G49010 | L13          | RPL13B    | Large subunit | 0.802 ± 0.155 |
| AT4G27090 | L14          | RPL14B    | Large subunit | 0.815 ± 0.200 |
| AT1G67430 | L17          | RPL17B    | Large subunit | 0.788 ± 0.185 |
| AT3G49910 | L26          | RPL26A    | Large subunit | 0.744 ± 0.077 |
| AT2G19730 | L28          | RPL28A    | Large subunit | 0.657 ± 0.236 |
| AT1G77940 | L30          | RPL30B    | Large subunit | 0.832 ± 0.112 |
| AT3G18740 | L30          | RPL30C    | Large subunit | 0.832 ± 0.107 |
| AT4G18100 | L32          | RPL32A    | Large subunit | 0.768 ± 0.145 |
| AT3G60245 | L37a         | RPL37aC   | Large subunit | 0.725 ± 0.114 |
| AT2G43460 | L38          | RPL38A    | Large subunit | 0.772 ± 0.127 |
differential intron retention in roots of Fe-deficient plants (Additional file 11). None of these RP genes were transcriptionally or translationally Fe-responsive.

**Comparative expression pattern of RP genes under Pi and Fe deficiency**

Comparison of the differentially expressed RP genes and RPs between Pi- and Fe-deficient Arabidopsis roots showed no overlap between the two growth types, suggesting that distinct RPs are involved in the responses of plants to Pi- and Fe-deficient. Interestingly, most RP genes encoding proteins of the large subunit were affected by Pi deficiency, while the majority of differentially RP genes encoding RPs under Fe deficiency were from both the small and the large subunit (Figure 5). Furthermore, the expression pattern of RPs upon Pi deficiency was more complex than that observed under Fe-deficient conditions. Nevertheless, one of the common observations for these two treatments is that there was a large discrepancy between the RP genes and PR proteins affected by Pi deficiency (compare Tables 1 and 2) as well as under Fe deficiency conditions (compare Tables 3 and 4). Our previous studies [35,49] have found that the concordance between differences in the abundance of mRNA and its encoded protein is not always high due to several factors: Firstly, proteins with low abundance might not be reliably detected and thus did not match the criteria that define them as differentially accumulated whereas the transcript levels of the corresponding genes are significantly changed. Secondly, several proteins with high abundance accumulated differentially upon Fe/Pi deficiency, but they have important housekeeping functions and the transcript levels were not changed. Finally, a change in transcript abundance may or may not be translated into changes in protein level; posttranscriptional regulatory processes such as alternative splicing, RNA processing, and other processes may affect the efficiency of translation. A generally low congruency of proteomic and transcriptional profiles has been reported previously [51].
| Gene ID     | Protein name | Gene name | Type      | Mean ± SD (−Fe/+Fe; P < 0.05) |
|------------|--------------|-----------|-----------|-----------------------------|
| AT1G72370  | Sα           | RPSαA     | Small subunit | 0.851 ± 0.094               |
| AT2G14840  | S2           | RPS2C     | Small subunit | 0.795 ± 0.057               |
| AT2G31610  | S3           | RPS3A     | Small subunit | 0.832 ± 0.012               |
| AT3G53870  | S3           | RPS3B     | Small subunit | 0.835 ± 0.051               |
| AT5G35530  | S3           | RPS3C     | Small subunit | 0.831 ± 0.059               |
| AT3G04840  | S3a          | RPS3aA    | Small subunit | 0.901 ± 0.046               |
| AT4G34670  | S3a          | RPS3aB    | Small subunit | 0.893 ± 0.066               |
| AT2G17360  | S4           | RPS4A     | Small subunit | 0.822 ± 0.083               |
| AT5G07090  | S4           | RPS4B     | Small subunit | 0.836 ± 0.057               |
| AT5G58420  | S4           | RPS4D     | Small subunit | 0.853 ± 0.057               |
| AT2G37270  | S5           | RPS5A     | Small subunit | 0.844 ± 0.028               |
| AT3G11940  | S5           | RPS5B     | Small subunit | 0.842 ± 0.065               |
| AT5G59240  | S8           | RPS8B     | Small subunit | 0.717 ± 0.159               |
| AT4G12160  | S9           | RPS9A     | Small subunit | 0.772 ± 0.053               |
| AT5G15200  | S9           | RPS9B     | Small subunit | 0.862 ± 0.050               |
| AT3G48930  | S11          | RPS11A    | Small subunit | 0.914 ± 0.027               |
| AT1G15930  | S12          | RPS12A    | Small subunit | 0.782 ± 0.008               |
| AT2G32060  | S12          | RPS12C    | Small subunit | 0.816 ± 0.080               |
| AT3G60770  | S13          | RPS13A    | Small subunit | 0.809 ± 0.086               |
| AT4G00100  | S13          | RPS13B    | Small subunit | 0.855 ± 0.003               |
| AT2G36160  | S14          | RPS14A    | Small subunit | 0.775 ± 0.043               |
| AT3G11510  | S14          | RPS14B    | Small subunit | 0.829 ± 0.050               |
| AT3G52580  | S14          | RPS14C    | Small subunit | 0.786 ± 0.124               |
| AT5G43640  | S15          | RPS15E    | Small subunit | 0.795 ± 0.063               |
| AT1G07770  | S15a         | RPS15aA   | Small subunit | 0.900 ± 0.054               |
| AT2G19720  | S15a         | RPS15aB   | Small subunit | 0.760 ± 0.040               |
| AT3G46040  | S15a         | RPS15aD   | Small subunit | 0.831 ± 0.034               |
| AT2G04390  | S17          | RPS17A    | Small subunit | 0.754 ± 0.016               |
| AT2G25220  | S17          | RPS17B    | Small subunit | 0.758 ± 0.012               |
| AT5G04800  | S17          | RPS17D    | Small subunit | 0.722 ± 0.019               |
| AT1G22780  | S18          | RPS18A    | Small subunit | 0.831 ± 0.019               |
| AT4G09800  | S18          | RPS18C    | Small subunit | 0.846 ± 0.028               |
| AT3G45030  | S20          | RPS20A    | Small subunit | 0.785 ± 0.071               |
| AT5G62300  | S20          | RPS20C    | Small subunit | 0.859 ± 0.042               |
| AT5G02960  | S23          | RPS23B    | Small subunit | 0.878 ± 0.032               |
| AT4G34555  | S25          | RPS23D    | Small subunit | 0.772 ± 0.082               |
| AT4G39200  | S25          | RPS25E    | Small subunit | 0.844 ± 0.066               |
| AT3G56340  | S26          | RPS26C    | Small subunit | 0.751 ± 0.058               |
| AT5G47930  | S27          | RPS27D    | Small subunit | 0.884 ± 0.075               |
| AT5G64140  | S28          | RPS28C    | Small subunit | 0.876 ± 0.033               |
| AT3G44010  | S29          | RPS29B    | Small subunit | 0.860 ± 0.049               |
| AT3G09200  | P0           | RPP0B     | Large subunit | 0.823 ± 0.103               |
| AT1G01100  | P1           | RPP1A     | Large subunit | 0.757 ± 0.042               |
| AT5G47700  | P1           | RPP1C     | Large subunit | 0.790 ± 0.049               |
In summary, our results suggest that the composition of ribosomes is changed in a stress-specific manner as part of an adaptation to Pi and Fe deficiency (and might be remodeled in other abiotic stresses), probably biasing protein translation (Figure 6). Our findings further suggest that RPs are not only involved in protein translation but also in the response of plants to nutrient deficiency,

### Table 3 Differentially expressed RP genes in roots of Fe-deficient plants (Continued)

| Protein ID   | Gene name | Type       | Fold change |
|--------------|-----------|------------|-------------|
| AT1G43170    | RPL3A     | Large subunit | 0.801 ± 0.051 |
| AT5G39740    | RPL5B     | Large subunit | 0.898 ± 0.042 |
| AT5G40130    | RPL5C     | Large subunit | 0.934 ± 0.054 |
| AT1G74060    | RPL6B     | Large subunit | 0.871 ± 0.067 |
| AT2G44120    | RPL7C     | Large subunit | 0.818 ± 0.019 |
| AT2G47610    | RPL7aA    | Large subunit | 0.858 ± 0.020 |
| AT3G62870    | RPL7aB    | Large subunit | 0.843 ± 0.060 |
| AT4G36130    | RPL8C     | Large subunit | 0.836 ± 0.081 |
| AT1G3120     | RPL9B     | Large subunit | 0.843 ± 0.033 |
| AT1G33140    | RPL9C     | Large subunit | 0.850 ± 0.018 |
| AT1G4320     | RPL10A    | Large subunit | 0.900 ± 0.017 |
| AT1G08360    | RPL10aA   | Large subunit | 0.838 ± 0.066 |
| AT2G27530    | RPL10aB   | Large subunit | 0.817 ± 0.063 |
| AT5G22440    | RPL10aC   | Large subunit | 0.861 ± 0.030 |
| AT2G42740    | RPL11A    | Large subunit | 0.793 ± 0.067 |
| AT3G58700    | RPL11B    | Large subunit | 0.761 ± 0.040 |
| AT5G23900    | RPL13D    | Large subunit | 0.860 ± 0.042 |
| AT3G07110    | RPL13aA   | Large subunit | 0.764 ± 0.009 |
| AT3G24830    | RPL13aB   | Large subunit | 0.731 ± 0.055 |
| AT4G13170    | RPL13aC   | Large subunit | 0.769 ± 0.066 |
| AT4G16720    | RPL15A    | Large subunit | 0.810 ± 0.031 |
| AT1G27400    | RPL17A    | Large subunit | 0.831 ± 0.073 |
| AT5G27850    | RPL18C    | Large subunit | 0.901 ± 0.029 |
| AT2G34480    | RPL18aB   | Large subunit | 0.787 ± 0.024 |
| AT3G14600    | RPL18aC   | Large subunit | 0.852 ± 0.022 |
| AT1G27800    | RPL19A    | Large subunit | 0.891 ± 0.040 |
| AT1G57660    | RPL21E    | Large subunit | 0.822 ± 0.055 |
| AT1G57860    | RPL21G    | Large subunit | 0.818 ± 0.045 |
| AT3G22330    | RPL27B    | Large subunit | 0.808 ± 0.067 |
| AT1G70600    | RPL27aC   | Large subunit | 0.819 ± 0.027 |
| AT2G19730    | RPL28A    | Large subunit | 0.872 ± 0.053 |
| AT1G77940    | RPL30B    | Large subunit | 0.867 ± 0.036 |
| AT5G46430    | RPL32B    | Large subunit | 0.901 ± 0.031 |
| AT5G02610    | RPL35D    | Large subunit | 0.905 ± 0.036 |
| AT1G18080    | RACK1A    | Other type gene | 0.758 ± 0.031 |
| AT1G48630    | RACK1B    | Other type gene | 0.840 ± 0.055 |
| AT3G56090    | FER3      | Other type gene | 0.511 ± 0.074 |

### Table 4 Differentially expressed ribosomal proteins in roots of Fe-deficient plants

| Protein ID   | Protein name | Gene name | Type       | Fold change |
|--------------|--------------|-----------|------------|-------------|
| AT3G05560    | RPL22        | RPL22B    | Large subunit | 0.5 ± 0.22 |
| AT5G27770    | RPL22        | RPL22C    | Large subunit | 0.58 ± 0.22 |

## Conclusions

In summary, our results suggest that the composition of ribosomes is changed in a stress-specific manner as part of an adaptation to Pi and Fe deficiency (and might be remodeled in other abiotic stresses), probably biasing protein translation (Figure 6). Our findings further suggest that RPs are not only involved in protein translation but also in the response of plants to nutrient deficiency,
possibly altering the composition of ribosomes in roots of Pi- or Fe-deficient plant. The functional divergence of RP paralogs could be attributable to genetically- or epigenetically-driven neofunctionalization and/or sub-functionalization, leading to highly specialized paralogs associated with Pi- and Fe-deficient processes. These findings may have important implications for understanding the molecular functions of RP genes in response to changing environmental conditions.

**Methods**

**Plant growth**

Arabidopsis (*Arabidopsis thaliana*) plants were grown in a growth chamber on an agar-based medium as described previously [52]. Seeds of the Col-0 accession were obtained from the Arabidopsis Biological Resource Center. Seeds were surface sterilized by immersing them in 5% (v/v) NaOCl for 5 min and 96% ethanol for 7 min, followed by four rinses in sterile water. Seeds were placed onto Petri dishes and kept for 1 d at 4°C in the dark; the plates were then transferred to a growth chamber and grown at 21°C under continuous illumination (50 μmol m^-2 s^-1; Philips TL lamps). The medium was composed of (mM): KNO₃ (5), MgSO₄ (2), Ca(NO₃)₂ (2), KH₂PO₄ (2.5); (μM): H₃BO₃ (70), MnCl₂ (14), ZnSO₄ (1), CuSO₄ (0.5), NaCl (10), Na₂MoO₄ (0.2); and 40 μM Fe-EDTA solidified with 0.3% Phytagel (Sigma-Aldrich). Suc (43 mM) and 4.7 mM MES were included, and the pH was adjusted to 5.5. After 10 d of pre-cultivation, plants were transferred either to fresh agar medium without Fe and with 100 μM 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine sulfonate, or medium without phosphate, or fresh control medium and grown for another 3 d. The lower concentration of potassium because of the absence of KH₂PO₄ in the phosphate-free medium was corrected by the addition of KCl.

**Data collection and data mining**

Transcriptomic data of roots from 13-d-old Arabidopsis seedlings grown in the presence or absence of Pi or Fe were downloaded from the NCBI SRA database (Pi: SRA050356.1; Fe: SRA045009) and analyzed as described in [30]. The proteomic data of Pi- or Fe-deficient Arabidopsis roots from 13-d-old Arabidopsis seedlings were
retrieved from [30] and [49], respectively. The cytosolic RP genes of *Arabidopsis thaliana* were compiled based on the Arabidopsis Genome Initiative (AGI) identification numbers provided by [4,32] (available in TAIR10).

**Gene ontology analysis**

Enrichment analysis of GO categories was performed with the Gene Ontology Browning Utility (GOBU) toolbox [53], using the TopGo ‘elim’ algorithm [54] for the aspects ‘biological process’ and ‘subcellular localization’. The selected categories were sorted from the lowest to the highest P value (P < 0.01).

**Generation of co-expression networks and modules of Fe-responsive ribosomal protein genes using the MACCU toolbox**

To generate root-specific networks of Pi- and Fe-responsive ribosomal protein genes, differentially expressed RP genes in Arabidopsis roots were identified using a Student’s t-test at a P value < 0.05. Gene networks were constructed based on 300 publicly available root-related microarray experiments using the MACCU toolbox as described in [44], with a Pearson’s correlation threshold of 0.7. The generated co-expression networks were visualized by Cytoscape (http://www.cytoscape.org). If one gene cluster did not have any connection (edges) to any other cluster in the co-expression network, we referred to such a cluster as module.

**Additional files**

| Additional file 1: List of RP genes and ribosome-related genes in the Arabidopsis genome. |
| Additional file 2: Expression of 247 RP and ribosome-related genes in Arabidopsis roots. |
| Additional file 3: 125 RPs identified in Arabidopsis roots. |
| Additional file 4: GO enrichment analysis of the 120 highly expressed RP genes in Arabidopsis roots. |
| Additional file 5: Gene lists of differentially expressed non-RP genes involved in the co-expression networks under Pi deficiency. |
| Additional file 6: Protein-protein interaction of 21 differentially expressed proteins under Pi deficiency in Arabidopsis roots. |
| Additional file 7: Differentially alternative splicing (DAS) features under Pi deficiency. |
| Additional file 8: GO enrichment analysis of the 81 differentially expressed RP genes under Fe deficiency. |
| Additional file 9: Co-expression relationships of 81 differentially expressed genes under Fe deficiency in Arabidopsis roots. |
| Additional file 10: Gene lists of differentially expressed non-RP genes involved in co-expression networks under Fe deficiency. |
| Additional file 11: DAS features under Fe deficiency. |

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

JYW, PL, and WL performed the data analysis and drafted the manuscript. HMG, LZ, and WS participated in the analysis of the data. PL and WL conceived and supervised the study. All authors approved the final version of the manuscript.
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