Unstable Transcripts in *Arabidopsis* Allotetraploids Are Associated with Nonadditive Gene Expression in Response to Abiotic and Biotic Stresses

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**Abstract**

Genome-wide analysis has documented differential gene expression between closely related species in plants and animals and nonadditive gene expression in hybrids and allopolyploids compared to the parents. In *Arabidopsis*, 15–43% of genes are expressed differently between the related species, *Arabidopsis thaliana* and *Arabidopsis arenosa*, the majority of which are nonadditively expressed (differently from mid-parent value) in allotetraploids. Nonadditive gene expression can be caused by transcriptional regulation through chromatin modifications, but the role of posttranscriptional regulation in nonadditive gene expression is largely unknown. Here we reported genome-wide analysis of mRNA decay in resynthesized *Arabidopsis* allotetraploids. Among ~26,000 annotated genes, over 1% of gene transcripts showed rapid decay with an estimated half-life of less than 60 minutes, and they are called allotetraploid genes with unstable transcripts (*AlloGUTs*). Remarkably, 30% of *AlloGUTs* matched the nonadditively expressed genes, and their expression levels were negatively correlated with the decay rate. Compared to all genes, these nonadditively expressed *AlloGUTs* were overrepresented 2-6-fold in the Gene Ontology (GOSlim) classifications in response to abiotic and biotic stresses, signal transduction, and transcription. Interestingly, the *AlloGUTs* include transcription factor genes that are highly inducible under stress conditions and circadian clock regulators that regulate growth in *A. thaliana*. These data suggest a role of mRNA stability in homoeologous gene expression in *Arabidopsis* allopolyploids. The enrichment of nonadditively expressed genes in stress-related pathways were commonly observed in *Arabidopsis* and other allopolyploids such as wheat and cotton, which may suggest a role for stress-mediated growth vigor in hybrids and allopolyploids.

**Introduction**

Polyploidy (whole genome duplication) is common in many plants and some animals [1,2,3]. The polyploid plants may represent over 70% of angiosperms [4,5], and >75% of which are allopolyploids [1,6,7]. Most important crops have a polyploidy origin. Wheat, cotton and canola are allopolyploid that consist of two or more divergent genomes [3], and some plants and animals exist as interspecific hybrids [8,9]. The common occurrence of plant polyploids indicates an advantage of being allopolyploids in response to selection and adaptation [6,10,11,12]. Stable allopolyploids with meiotic transmission of homoeologous genomes may lead to permanent fixation of heterozygosity and hybrid vigor.

Stable *Arabidopsis* allotetraploids (*Arabidopsis suecica*-like) are resynthesized by crossing tetraploid *Arabidopsis thaliana* with *Arabidopsis arenosa* [13]. The resynthesized *A. suecica* allotetraploids show morphological and physiological differences from their progenitors [13,14,15]. Microarray analysis of gene expression indicated that 5–38% of the genes are nonadditively expressed (different from mid-parent value, MPV) in resynthesized allotetraploids [14]. Nonadditive expression suggests activation or repression of a gene through genetic and epigenetic mechanisms [16,17,18]. Gene activation or silencing in allotetraploids can be caused by transcriptional regulation through changes in chromatin structure. However, many expression assays including microarrays and RNA sequencing measure the steady levels of mRNA transcripts. It is unknown whether mRNA stability affects nonadditive expression of genes between allotetraploids and their progenitors, *A. thaliana* and *A. arenosa*.

Posttranscriptional regulation of mRNA plays an important role in gene expression through modulation of mRNA stability and turnover. For example, *c-myc* and *c-fos* transcripts in mammalian cells and mating-type transcripts in yeast are unstable [19,20,21]. The decay rate of mRNA is correlated with biological functions of the genes and the length of cell cycles in mammals and yeast [22,23,24]. There is a role for mRNA stability in shaping the kinetics of the transcriptome in response to environmental changes in yeast and mammals [25,26]. Approximately 50% of stress responsive genes in human cells are controlled at the level of mRNA decay [27]. In yeast, mRNA turnover is a major mediator in the response to heat shock, nutrition deprivation, oxidative stress, and osmotic stress [22,28,29].

Genome-wide analysis of mRNA decay was studied in *Arabidopsis* [30,31]. It is shown approximately 1% of *Arabidopsis*
thaliana genes with unstable transcripts (AtGUTs) [30]. These AtGUTs include the genes that are induced by mechanical stimulation and circadian rhythms. Moreover, transcripts from intronless genes and microRNA target genes generally have short half-lives [31]. Unstable transcripts are also identified in photo-labile phytochrome genes [32] and auxin inducible genes [33,34], which facilitates rapid turnover of mRNA, leading to tight and effective control of gene expression [35]. To test a role of mRNA stability in nonadditive gene expression in allopolyploids, we examined mRNA turnover rates in resynthesized A. suecica allopolyploids relative to the mid-parent value using oligo-gene microarrays [14]. The candidates of allopolyploid genes with unstable transcripts (AlloGUTs) were compared with the genes that are nonadditively expressed, as identified in a previous study [14].

We further analyzed Gene Ontology (GOSlim) classifications and compared AlloGUTs with stress responsive genes in published microarray datasets. Finally, we validated expression for a subset of genes using qRT-PCR analysis. These comprehensive analyses revealed mRNA instability of the genes related to stresses and clock-mediated pathways, suggesting a role for rapid mRNA decay in regulating stress and circadian responses to growth vigor in allopolyploids.

Results

Genome-wide analysis of unstable transcripts in allopolyploids using oligo-gene microarrays

Spotted oligo-gene microarrays were used to evaluate mRNA turnover in allopolyploids relative to the MPV (Figure 1A). The 70-mer oligos were designed from annotated genes and could cross-hybridize with A. thaliana and A. arenosa genes. This may underestimate changes in allopolyploids in which transcripts from one homoeologous locus display rapid decay, whereas the transcripts from the other locus are relatively stable. After data normalization and statistical tests (see Methods), the qualified values with reproducibly normalized intensity ratios of ≥ 2 were used for comparative analysis of gene expression. Approximately ~1% (200) of genes in the Arabidopsis allotetraploid (Allo733) were identified as unstable transcripts with a half-life time of less than 60 min using the same criteria as previously defined for unstable transcripts [30].

These are hereafter referred to as allotetraploid genes with unstable transcripts (AlloGUTs) (Table S1). The percentage of AlloGUTs identified in allopolyploids is consistent with ~1% of AtGUTs identified in A. thaliana using cDNA microarrays [30]. To confirm the microarray data, a subset of 10 genes was validated using quantitative RT-PCR (qRT-PCR) analysis, and expression patterns of four AlloGUTs were determined (Figure 1B, C). The four genes are: At5g26030 (ATFC1) encoding ferrochelatase 1, At4g17500 (ERF1) encoding ethylene responsive element binding factor 1, At1g27730 (ZAT10) encoding salt tolerance zinc finger, and At4g11280 (ACS6) encoding 1-aminocyclopropane-1-carboxylic acid (ACC) synthase 6. In qRT-PCR analysis, the gene encoding translation initiation factor 4A2 (eIF4A2, At1g54270) was used as a control because its transcripts were reasonably stable and did not degrade during the time course of chemical treatments [30]. The estimated turnover rate was 24–40 minutes by qRT-PCR (Figure 1C), which was comparable to 29–47 minutes estimated from microarray data (Figure 1B).

Figure 1. Identification of unstable transcripts with a half-life time of less than 60 min in Arabidopsis allotetraploids and validation of microarray data. (A) Experimental procedure for monitoring mRNA decay rates in Arabidopsis allotetraploids using spotted 70-mer oligo-gene microarrays. (B) qRT-PCR analysis (open bar) of half-life time of four genes with unstable transcripts identified by microarrays (hatched bar). (C) Quantification of mRNA decay rates of four genes and estimation of their half-life time.

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A total of ~208 AlloGUTs and ~95 AtGUTs were classified into 20 GO Slim groups (Figure 2) using TAIR release 9 (November 2009) and PEDENT (http://mips.gsf.de/proj/thal/db/index.html). The proteins encoded by AlloGUTs were distributed among all GO Slim biological classifications. Compared with the proportion of the whole-genome annotated genes, AlloGUTs were overrepresented by a factor of two in the groups of cell cycle and DNA processing, transcription, regulation of metabolism and protein, and cellular communication and biogenesis of cellular component (Figure 2). In particular, the genes with unstable transcripts were enriched in four groups, namely, cell rescue, defense and virulence, interaction with environment, and systemic interaction with environment. They had significantly (2.6–3.2-fold) higher proportions of GUTs than that of all genes (dashed lines) [30] (Figure 2).

Rapid mRNA turnover and nonadditive gene expression in allotetraploids

Unstable transcripts are associated with mRNA stability and possibly steady levels of transcripts in allotetraploids. To test this, we compared the gene list of nonadditively expressed genes identified in a previous study [14] with that of AtGUTs in A. thaliana diploid [30] and AlloGUTs in this study. Sixty-one of 208 (~30%) AlloGUTs matched the nonadditively expressed genes in both allotetraploids (Allo733 and Allo738) (Table 1, Figure 3A). The correlation was statistically significant (degree of freedom = 1, Chi-square = 460.945, and p<0.01) (Table S2). Hierarchical cluster analysis suggested a strong negative correlation between the expression levels of nonadditively expressed genes and mRNA decay rates of AlloGUTs (Figure 3B). The data suggest that these nonadditive expressions of the genes in the allotetraploids tested are related to rapid mRNA turnover. We also examined an enrichment of these genes in biological processes compared with the whole genome annotation. As in the nonadditively expressed genes [14,36], the nonadditively expressed AlloGUTs were enriched 2–3 fold in GO Slim groups of stress response pathways, followed by response to abiotic or biotic stimulus, signal transduction, and transcription (Figure S1).

A relationship between nonadditively expressed AlloGUTs and abiotic stress and phytohormone responses

To test how nonadditively expressed AlloGUTs are affected by physiological and environmental conditions, we performed hierarchical cluster analysis with the Genevastor program [37,38], using the genes extracted from multiple expression datasets, including global stress [38] and response to hormones and hormone-like chemicals [37]. The comparative data analysis indicated that nonadditively expressed AlloGUTs matched the genes whose expression was highly induced by cold, drought, osmotic, oxidative, salt and wounding (Figure 4). In addition, the nonadditively expressed AlloGUTs were also highly induced by ozone stress (Figure 5) and tend to alter expression levels in response to the treatments of phytohormones and plant hormone substances, ABA and Methyl Jasmonic acid, cyclohexamide and AgNO3 (Figure S2).

Interestingly, under normal growth conditions these stress responsive genes had a fast decay rate, and nonadditively expressed genes were down-regulated in the allotetraploids. This suggests stress-related genes are temporally repressed in allotetraploids under normal conditions and may be inducible under stress conditions.

Nonadditively expressed AlloGUTs also show a nonadditive mRNA decay rate

To test whether nonadditively expressed AlloGUTs are subjected to nonadditive mRNA decay in the allotetraploids, we measured...
Table 1. List of nonadditively expressed AlloGUTs.

| Locus      | Description                                                                 | t1/2(min) |
|------------|-----------------------------------------------------------------------------|-----------|
| AT5G62410  | SMC2 (STRUCTURAL MAINTENANCE OF CHROMOSOMES 2)                               | 16.86     |
| AT3G44260  | CCR4-NOT transcription complex protein, putative                            | 18.81     |
| AT3G02550  | LBD41 (LOB DOMAIN-CONTAINING PROTEIN 41)                                     | 19.45     |
| AT3G10040  | transcription factor                                                         | 20.54     |
| AT3G15210  | ATERF-4 (ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 4)                       | 21.72     |
| AT1G18740  | similar to unknown protein                                                   | 21.87     |
| AT2G28200  | nucleic acid binding/transcription factor/zinc ion binding                   | 23.28     |
| AT2G44500  | similar to unknown protein                                                   | 23.38     |
| AT1G28370  | ATERF11/ERF11 (ERF domain protein 11)                                        | 24.06     |
| AT4G11280  | AC36 (1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC) SYNTHASE 6)                | 24.15     |
| AT4G15760  | DL3920C, FCAALL.376, M01, MONOOXYGENASE 1                                   | 24.71     |
| AT5G63160  | BTB and TAZ domain protein                                                   | 25.00     |
| AT2G22880  | VQ motif-containing protein                                                  | 25.08     |
| AT2G31880  | leucine-rich repeat transmembrane protein kinase, putative                  | 25.08     |
| AT1G02400  | ATGA2OX6/DTA1 (GIBBERELLIN 2-OXIDASE 6)                                      | 25.14     |
| AT4G27280  | calcium-binding EF hand family protein                                       | 25.36     |
| AT1G72520  | lipoxigenase, putative                                                       | 25.51     |
| AT1G18300  | ARABIDOPSIS THALIANA NUDIX HYDROLASE HOMOLOG 4, ATNUDT4,                     | 25.56     |
| AT5G22250  | CCR4-NOT transcription complex protein, putative                            | 25.70     |
| AT3G15210  | ATERF-4(ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 4)                        | 26.04     |
| AT5G17950  | unknown protein                                                             | 26.05     |
| AT3G01830  | calmodulin-related protein                                                   | 26.98     |
| AT1G68840  | RAV2 (REGULATOR OF THE ATPASE OF THE VACUOLAR MEMBRANE)                     | 27.68     |
| AT1G72950  | disease resistance protein (TIR-NBS class), putative                        | 27.98     |
| AT2G28400  | similar to unknown protein                                                   | 28.02     |
| AT2G41640  | similar to unknown protein                                                   | 28.34     |
| AT1G72430  | auxin-responsive protein-related                                            | 28.60     |
| AT3G52450  | U-box domain-containing protein                                              | 28.71     |
| AT1G56510  | disease resistance protein (TIR-NBS-LRR class), putative                    | 28.72     |
| AT3G19580  | AZF2 (ARABIDOPSIS ZINC-FINGER PROTEIN 2)                                     | 28.96     |
| AT1G17380  | JAZS/TIFY11A (JASMONATE-ZIM-DOMAIN PROTEIN 5)                                | 29.00     |
| AT2G0670  | ATNUDT17 (Arabidopsis thaliana Nudix hydrolase homolog 17)                   | 29.47     |
| AT5G63790  | ANAC102 (Arabidopsis NAC domain containing protein 102)                     | 30.14     |
| AT1G27730  | STZ (SALT TOLERANCE ZINC FINGER)                                             | 30.20     |
| AT1G66160  | U-box domain-containing protein                                              | 30.21     |
| AT4G18950  | ankyrin protein kinase, putative                                             | 30.72     |
| AT5G63450  | CYP94B1 (cytochrome P450, family 94, subfamily B, polypeptide 1)             | 30.85     |
| AT2G42760  | similar to unnamed protein product [Vitis vinifera]                          | 30.94     |
| AT2G38470  | WRKY33 (WRKY DNA-binding protein 33); transcription factor                 | 31.65     |
| AT1G76600  | similar to unknown protein                                                   | 31.94     |
| AT4G33050  | EDA39 (embryo sac development arrest 39); calmodulin binding               | 32.48     |
| AT1G20510  | OPCL1 (OPC-8:0 COA LIGASE1); 4-coumarate-Coa ligase                         | 33.50     |
| AT4G17500  | ATERF-1 (ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 1)                       | 34.02     |
| AT5G11650  | hydrolase, alpha/beta fold family protein                                    | 34.06     |
| AT4G08170  | inositol 1,3,4-trisphosphate 5/6-kinase family protein                     | 34.12     |
| AT1G27100  | similar to unknown protein                                                   | 34.26     |
| AT5G13220  | JAS1/JAZ10/TIFY9 (JASMONATE-ZIM-DOMAIN PROTEIN 10)                          | 34.77     |
| AT2G41140  | calmodulin, putative                                                        | 35.20     |
| AT1G44350  | ILL6 (IAA-leucine resistant (ILR)-like gene 6); metalloproteinase          | 35.55     |
| AT4G24380  | hydrolase, acting on ester bonds                                            | 36.05     |
Among 61 genes tested, 14 (∼25%) of nonadditively expressed *AlloGUTs* were significantly more unstable than the MPV (Figure 6). The majority of 14 genes tested, except for AT3g44260, had a shorter half-life time in the allotetraploid than MPV (Figure 6). According to functional classification by PEDENT, eight genes (∼57%) [At1g27730, At1g68840, At2g28290, At3g15210, At3g15500, At3g19580, At3g41260, and At4g17500] were predicted with a role in transcription. For example, AtNAC3 (At3g15500) is one of the nonadditively expressed *AlloGUTs*, and its expression is sensitive in response to a wide range of stresses and phytohormones. This transcript had a much shorter half-life in the allotetraploid than in the progenitors. Furthermore, hierarchical cluster analysis indicated that nonadditively expressed *AlloGUTs* are highly correlated with the genes in response to abiotic stress and phytohormones. The data suggest that nonadditive gene expression in the allotetraploids is associated with the nonadditive rate of mRNA decay and stress and phytohormone responses.

**Table 1. Cont.**

| Locus          | Description                                                                 | t1/2(min) |
|---------------|-----------------------------------------------------------------------------|-----------|
| AT1G09940     | HEMA2: glutamyl-tRNA reductase                                               | 36.53     |
| AT2G01180     | ATPAP1 (PHOSPHATIDIC ACID PHOSPHATASE 1)                                     | 37.57     |
| AT3G10930     | similar to unknown protein (*Arabidopsis thaliana*) (TAIR:AT3G05300.1)       | 37.98     |
| AT3G25780     | AOC3 (ALLENE OXIDE CYCLASE 3)                                               | 38.15     |
| AT1G16370     | ATOCT6; carbohydrate transmembrane transporter                              | 38.17     |
| AT5G54170     | similar to CFT (*Arabidopsis thaliana*) (TAIR:AT1G64720.1)                  | 38.17     |
| AT5G05600     | oxidoreductase, 2OG-FelH family protein                                       | 39.72     |
| AT2G35460     | harpin-induced family protein/HIN1 family protein                           | 40.12     |
| AT3G50950     | disease resistance protein (CC-NBS-LRR class), putative                   | 40.73     |
| AT3G15500     | ATNAC3 (*Arabidopsis NAC DOMAIN CONTAINING PROTEIN 55)                      | 40.75     |
| AT5G26030     | FC1 (FERROCHELATASE 1); ferrochelatase                                       | 40.95     |

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the difference in mRNA decay rates between allotetraploids and their progenitors, *A. thaliana* and *A. arenosa*, using qRT-PCR (Figure 6). Among 61 genes tested, 14 (∼25%) of nonadditively expressed *AlloGUTs* were significantly more unstable than the MPV (Figure 6, Table 1). The majority of 14 genes tested, except for At3g44260, had a shorter half-life time in the allotetraploid than MPV (Figure 6). According to functional classification by PEDENT, eight genes (∼57%) [At1g27730, At1g68840, At2g28290, At3g15210, At3g15500, At3g19580, At3g41260, and At4g17500] were predicted with a role in transcription. For example, AtNAC3 (At3g15500) is one of the nonadditively expressed *AlloGUTs*, and its expression is sensitive in response to a wide range of stresses and phytohormones. This transcript had a much shorter half-life in the allotetraploid than in the progenitors. Furthermore, hierarchical cluster analysis indicated that nonadditively expressed *AlloGUTs* are highly correlated with the genes in response to abiotic stress and phytohormones. The data suggest that nonadditive gene expression in the allotetraploids is associated with the nonadditive rate of mRNA decay and stress and phytohormone responses.

**Different decay rates of circadian clock gene transcripts in allotetraploids**

Nonadditively expressed genes in allotetraploids include those encoding circadian clock regulators, including CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), LATE ELONGATED HYPOCOTYL (LHY), TIMING OF CAB EXPRESSION 1 (TOC1), and GIGANTEA (GI) [14,39,40,41,42]. The regulation of *CCA1* transcripts by light and its mRNA instability play a role for accurately entraining the oscillator in response to environmental changes [43]. Indeed, we found CCA1 binding motifs and evening elements within 1000-bp upstream region of nonadditively expressed *AlloGUTs*. Among 14 *AlloGUTs*, 7 genes (50%) each contains at least one CCA1 binding site (CBS: AAAAAATCT) or evening element ([AA/AATATCT], [39,44], suggesting that these genes are likely the targets of CCA1 and LHY. We measured the stability of *CCA1*, *LHY*, *TOC1*, and GI mRNA in allotetraploid and its progenitors (Figure 7). Transcripts of *CCA1* and *LHY* were relatively unstable, as previously reported [43], whereas the transcripts of their reciprocal regulators, *TOC1* and GI, were relatively stable. The decay rate of *CCA1* mRNA in the allotetraploid is relatively faster than MPV, and *LHY* mRNA decreased faster in the allotetraploid than in one of the progenitors. These two nonadditively expressed clock regulator genes also showed a nonadditive rate of mRNA decay.

**Discussion**

**Posttranscriptional regulation of cell cycle and stress-related genes in Arabidopsis allotetraploids**

Transcriptome divergence between allotetraploids and their parents are controlled by transcriptional and/or post-transcriptional regulation [16,18]. At the transcriptional level, chromatin modifications and DNA methylation mediate activation and silencing of homoeologous genes in allotetraploids or interspecific hybrids [14,15,45,46,47]. Changes in transcript abundance can also be regulated at the posttranscriptional level. In synthesized wheat allotetraploids, activation of retrotransposon affects the expression of neighboring genes [48]. RNA-mediated gene expression may be associated with the equilibrium between mRNA accumulation and stability during growth, development, and physiological transitions [49,50,51,52,53]. Here we provided the first evidence for mRNA instability study in nonadditive gene expression in *Arabidopsis* allotetraploids. Our data indicated that the genes with unstable transcripts are correlated with nonadditive levels of steady mRNAs in the allotetraploids. Therefore, a proportion of nonadditively expressed genes in the allotetraploids is caused by unstable transcripts.

The relatively small number of *alloGUTs* identified in the study can be due to several factors. One possibility is that unstable mRNAs have low steady state levels, which may not be detectable by microarrays. For example, circadian clock genes such as *CCA1* and *LHY* have unstable transcripts [43] but were not identified by microarrays. Alternatively, cross-hybridization of the probes between species could be another factor that have under-estimated the number of genes with unstable transcripts. One might expect that additional *GUTs* will be identified using high-resolution next-generation sequencing technology (RNA-seq) [54]. Alternatively, the noise associated with quantification of mRNA stability may also account for some differences observed.

**GO Slim classifications show that like AtGUTs, AlloGUTs are found in all plant cellular processes. The proportion of AlloGUTs is enriched 2-fold or more that of all genes in several GO groups, including transcription, cell cycle and DNA processing,**
mRNA Instability in Arabidopsis Allopolyploids

and external signals such as biotic and abiotic stresses [30,31]. The data are consistent with high retention rates of duplicate genes and high expression divergence between duplicate genes than single-copy genes in the process of polyploidization [36,57]. Nonadditive expression of stress-related genes is also overrepresented in other allopolyploids such as wheat [38,59] and cotton [60,61,62]. This may also suggest that \textit{AlloGUTs} are involved in these processes to facilitate faster mRNA adjustment in allopolyploids and hybrids than in diploids [21,24,30,35]. During evolution, rapid mRNA turnover of the transcripts made from additional genes in allopolyploids is likely to facilitate selection and adaptation for environmental niches [6].

\textit{AlloGUTs} are associated with nonadditive gene expression in allotetraploids in response to stresses and phytohormones

A significant finding is that \textasciitilde 30\% of \textit{AlloGUTs} overlap with the nonadditively expressed genes in allotetraploids [14] (Figure 3A). Moreover, there is a statistically significant negative correlation between the expression levels of \textit{AlloGUTs} and nonadditively expressed genes in the allotetraploids. The unstable mRNA transcripts tend to be nonadditively repressed in allopolyploids, suggesting a role of mRNA instability in nonadditive gene expression in allopolyploids. In other words, different transcriptome profiles of nonadditively regulated genes can be generated by different sensitivities of the mRNA degradation rate during allopolyploidization.

The \textit{AlloGUTs} that overlap with nonadditively expressed genes are enriched 2–7-fold in GOslim groups in response to stress, abiotic or biotic stimulus, signal transduction and transcription. There is also a 1.5-fold enrichment of the GO groups in transcription, developmental processes, other cellular processes and other metabolic processes. Compared to additively expressed \textit{AlloGUTs}, nonadditively expressed \textit{AlloGUTs} are significantly correlated with the genes in GOslim groups in response to cold, drought, osmotic, salt, and wounding stress. In response to environmental stimuli, an increase or decrease in mRNA abundance might be attained either by adjusting the rate of transcription or the rate of degradation, as demonstrated in many studies [25]. In addition, nonadditively expressed \textit{AlloGUTs} are correlated with the genes induced by Methyl jasmonic acid and plant hormone substances, such as AgNo3, cycloheximide, and 2,3,5-triiodobenzoic acid. In cotton allopolyploids, expression ratios of \textit{AdhA} homologs vary considerably during seedling and fruit development [62]. Homoeologous gene expression is altered by abiotic stress treatments, including cold, dark, and water submersion. These data collectively suggest that nonadditive gene expression and unstable transcripts provide a general mechanism in response to external signals such as biotic and abiotic stress as well as to internal signals such as phytohormones and intergenomic interactions.

Nonadditively expressed \textit{AlloGUTs} also show nonadditive rate of mRNA decay in both parents. Among them, 25\% of \textit{AlloGUTs} have a faster decay rate in the allotetraploid than in the parents, 57\% of which encode transcription factors. One gene, \textit{AtNAC3} (At3g15500), encodes one member of the NAC transcription factor family. The expression of \textit{AtNAC3} is highly induced by drought, high salinity, and abscisic acid [63]. Genome-wide analysis of \textit{AtNAC3}-overexpressing transgenic plants showed a significant increase in drought tolerance, which was correlated with upregulation of several stress-inducible genes. The transgenic plants overexpressing \textit{AtNAC3} also show significantly higher survival rate (100\%) under drought stress [63]. Another gene, \textit{AtERF1} (At4g1750), encodes ETHYLENE RESPONSE FACTOR1, a transcription factor, which controls the expression of pathogen responsive genes and prevents disease progression.

Figure 3. A relationship between \textit{AlloGUTs} and nonadditively expressed genes in allotetraploids (Allo733 and Allo738). (A) Venn diagrams showing overlapping genes between \textit{AlloGUTs} with half-life time less than 60 min and the common set of nonadditively expressed genes in both Allo733 and Allo738. (B) Heat map constructed from mRNA decay rates of \textit{AlloGUTs} (first column, mainly red) and expression levels of nonadditively expressed genes (second column, mainly green). The bar shows relative levels of log2-fold changes (from \textasciitilde3 to \textasciitilde3).

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cellular communication, and biogenesis of cellular components. The majority of these genes are involved in cell cycle regulation and DNA metabolism, a process that requires rapid and cyclic regulation. For example, in yeast, cell cycle and mating-type genes have unstable transcripts [22,24,55,56]. Significantly, \textit{AlloGUTs} were enriched 3-fold more transcripts that are related to exogenous stimulus such as cell rescue, defense and virulence and interaction with the environments (Figure 2), suggesting that these genes are regulated by the rate of mRNA decay. Rapid decay of mRNA is a mechanism for fast response to environmental conditions.
Figure 4. Hierarchical cluster analysis showing the expression levels of the genes induced by stress that matched AlloGUTs in allotetraploids. The analysis was carried out using publicly available microarray database (http://www.arabidopsis.org/portals/expression/microarray/ATGenExpress.jsp) and Genevastor. Each row represents log2 expression values of the genes in a given microarray experiment, and each column represents a gene. The color represents relative expression levels of the genes (from $-3$ green to $+3$ red).

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Overexpressing AtERF1 in the transgenic plants increases resistance to pathogens [64], as well as complements defense response defects of coronatine insensitive 1 (coi1) and ethylene insensitive2 (ein2) mutants [65]. Interestingly, the transgenic plants overexpressing AtERF1 are significantly smaller than wild-type plants. The above data are consistent with the observation that many stress responsive genes are generally repressed in the allotetraploids under normal conditions [14]. Allotetraploids might have developed a mechanism that requires low maintenance at the transcript level for these genes in response to environmental changes. Under stress conditions, adjusting the ratio of unstable and stable mRNA transcripts may help rapidly facilitate the proper mRNA levels in response to stress. Whether or not the regulation of stress responsive genes is correlated with increased growth vigor in allopolyploids remains to be investigated.

Circadian clock genes produce unstable transcripts in allotetraploids and their progenitors

Plants are sessile and need to prepare for and respond to all environmental changes including biotic and abiotic stresses. Recent studies suggested that 10–90% genes in Arabidopsis are governed by circadian clock regulation [40,44,66,67]. CCA1 and LHY are members of a small family of single Myb domain transcription factors and have redundant but non-overlapping functions. CCA1 and LHY bind to the promoter of TOC1 and downstream genes in photosynthesis and starch metabolism [39,44]. CCA1 expression is negatively regulated by TOC1 and CHE [42,68]. Accurate entrainment of circadian rhythms is controlled by a combination of CCA1 transcripts level and mRNA degradation by light [43]. We found that CCA1 and LHY transcripts are unstable and show a relatively high rate of mRNA turnover. Compared to CCA1 and LHY, TOC1 and GI transcripts are relatively stable. Interestingly, these clock genes are nonadditively expressed in allotetraploids [14,39], and their expression levels are negatively correlated with their decay rates (this study). Specifically, CCA1 and LHY mRNA has a relatively lower level of stability in the allotetraploid than in both parents. The data suggest in addition to transcriptional regulation, some circadian clock genes such as CCA1 and LHY are regulated through mRNA decay at the posttranscriptional level. Together, the available data indicate that transcriptional and posttranscriptional regulation of circadian clock genes, stress responsive genes, and many other GUTs provides a general mechanism for rapid changes in gene expression induced by “genome shock” [69] in response to interspecific hybridization and allopolyploidization. Posttranscriptional regulation of mRNA transcripts offers a unique mechanism to discriminate the transcripts between homoeologous loci in the allopolyploids. It will be interesting to investigate how these homoeologous transcripts are discriminated and degraded.

Conclusions

The current data support a role of mRNA instability in nonadditive expression of genes in Arabidopsis allotetraploids. We found ~1% of genes with allotetraploid unstable transcripts (AlloGUTs) in Arabidopsis and a half-life time of less than 60 minutes, ~30% of which matched the nonadditively expressed genes that are repressed in the allotetraploids. All AlloGUTs and
nonadditively repressed AlloGUTs are significantly enriched with GO Slim classifications of response to stress, environment, and phytohormones. These AlloGUTs overlap with the genes that are highly induced by abiotic stress, including cold, drought, osmotic, salt, and wounding, and plant hormones and substances, including Methyl jasmonic acid and cycloheximide. Expression of circadian clock genes such as CCA1 and LHY is controlled by transcriptional regulation as well as posttranscriptional regulation through mRNA decay. The data collectively suggest that transcriptional and posttranscriptional regulation affects expression of homoeologous genes related to stress response, light signaling, and hormone interactions in allopolyploids, which may provide a flexible and rapid response to external and internal stimuli as a consequence of polyploidization.

Materials and Methods

Plant Material and RNA sample preparation for half-life time calculation

All plants were grown under sterile conditions on plates containing 1x Murashige and Skoog salts, 1x Gamborg’s vitamins and 1% sucrose for 2 weeks at 22°C. Light regime was 16-hr light and 8-hr dark in a growth chamber. Plant materials included A. thaliana autotetraploid (At4, accession no. CS3900, 2n = 4x = 20), tetraploid A. arenosa (Aa, accession no. CS3901, 2n = 4x = 32), and resynthesized A. suecica allotetraploid lines in F8 generation (Allo733 and 738, 2n = 4x = 26, accession no. CS3895-3896) [13,14,15]. Two-week old seedlings were used for all analyses.

The plants were incubated in a beaker containing incubation buffer for 30 min [32]. After the incubation, 3’-deoxyadenosine (cordycepin) was added to a final concentration of 200 mg/ml for all seedlings. Cordycepin was used as a transcription inhibitor because it works better than other inhibitors such as Actinomycin D and it is more effectively to penetrate into leaf tissues [30,32,70]. Treated leaves were harvested at each time point, 0, 60, and 120 min, after inhibitor treatment, and immediately frozen in liquid nitrogen. Total RNA was isolated using Plant RNA reagent (Invitrogen, Carlsbad, CA). Each RNA sample was quantified by measuring the 260/280 ratio using Nano drop and by agarose-formaldehyde gel electrophoresis. Quantified total RNA was subjected to mRNA isolation by Micro-fast Track 2.0 mRNA isolation kit (Invitrogen). Isolated mRNA was reverse-transcribed to synthesize cDNA using Cy3- or Cy5- labelled dCTPs (Amersham Biosciences, Piscataway, NJ) that was used as probes for microarray analysis, as previously described [14].

Quantitative RT-PCR

Approximately 5 mg of total RNA treated with DNase I was reverse-transcribed using SuperScriptII (Invitrogen), following the manufacturer’s recommendation. The synthesized cDNA was diluted 1:5 in DEPC water and subjected to quantitative RT-PCR (qRT-PCR) analysis using SYBR Green Supermix (ABI Biosystems, Foster City, CA) in an ABI 7500 instrument (ABI Biosystems). The qRT-PCR conditions were optimized to maximize the amplification efficiency as previously described [71]. In this study, the gene eIF4A2 was used as an internal control to estimate the relative transcript level of the gene tested. The primers were provided in Table S3.

Spotted oligo-gene microarrays and data analysis

Spotted oligo-gene microarrays with 26,090 annotated genes were used for monitoring mRNA stability in allotetraploids according to the published protocol [14,71,72]. A total of 16 slides were used for two-time course comparisons (0 min vs. 120 min), including two biological and two technical replications. Each biological replicate consists of two dye-swaps (four slides) that were hybridized with reverse-labeled probes using one mRNA sample from the allotetraploid and another mRNA sample from a...
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mix of RNAs from two parents (mid-parent value, MPV) (Figure 1A). For microarray hybridization, 500 ng of mRNA were used to synthesize cDNA in each labelling reaction using Cy3- or Cy5-dCTP (Amersham Biosciences, Piscataway, NJ). Probe labelling, slide hybridization, and washing were according to a published protocol [72]. After the slides were scanned using Genepix 4000B, raw data were collected using Genepix Pro4.1. The replicated data from the dye-swap of each comparison were extracted and normalized using Acuity 4.0 software (Molecular Devices, http://www.moleculardevices.com). Quantified values in each dataset were analyzed by t-test using a statistical significance level of α = 0.001. The common genes between the two datasets with reproducibly normalized intensity ratios of ≥2 were used for further analysis. The difference of the transcript abundance between each time point can be used to calculate the half-life by the equation ln (Normalized Ratio) = -t1/2/kd, with t1/2 = 0.693/ kd, since mRNA degradation generally obeys first-order kinetics, which is common in fast mRNA degradation [30,73]. Gene Ontology (GOSlim) of AlloGUTs was classified using TAIR release [November 2009] (http://ftp.arabidopsis.org/home/tair/ Ontologies/Gene_Ontology/ATH_GO_GOSLIM.txt) and PEDANT (http://mips.gsf.de/proj/thal/db/index.html). Clustering analysis of gene expression was performed using CLUSTER and TREEVIEW [74] (http://rana.lbl.gov/EisenSoftware.htm). Our microarray data were comparatively analyzed with the published data [14] to determine if nonadditively expressed AlloGUTs were enriched in GOSlim groups in response to stress [38], hormones [37], and circadian regulation [75]. The data were downloaded from http://www.arabidopsis.org/portals/expression/microarray/ ATGenExpress.jsp. Visual display of expression profiles from multiple experiments was performed in Genevestigator [76] (https://www.genevestigator.com/gv/index.jsp). All microarray data are MIAME compliant http://www.ncbi.nlm.nih.gov/geo/info/MIAME.html and deposited in GEO http://www.ncbi.nlm.nih.gov/geo/ with the accession GSE26065.

Supporting Information

Figure S1 The percentages of the genes in GOSlim category that are nonadditively expressed AlloGUTs (61 genes), compared to all AlloGUTs. The ratios in the y-axis were calculated using the observed percentage of the nonadditively expressed AlloGUTs divided by the expected percentage of all annotated genes in the Arabidopsis genome. The dashed line shows the observed percentage of the genes that are AlloGUTs in a microarray experiment equal to that of the expected genes in the whole genome (100%).

Figure S2 Hierarchical cluster analysis showing AlloGUTs that matched the genes whose expression is induced by hormone and chemical treatments. The analysis was carried out with publicly available microarray database (http://www.arabidopsis.org/portals/expression/microarray/ ATGenExpress.jsp and Genevestor). Each row represents a microarray experiment and each column represents a gene. The color represents the relative expression level of each experimental group (Red, up and green, down).

Table S1 Arabidopsis allotetraploid genes with unstable transcripts (AlloGUTs).

Table S2 Statistic tests for significance between microarray datasets.

Table S3 Primer sequences used in the analysis.

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Author Contributions

Conceived and designed the experiments: ZJC E-DK. Performed the experiments: E-DK. Analyzed the data: E-DK. Contributed reagents/materials/analysis tools: E-DK. Wrote the paper: ZJC E-DK.

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