Impact of natural genetic variation on the transcriptome of autotetraploid *Arabidopsis thaliana*

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Polyploidy, the presence of more than two complete sets of chromosomes in an organism, has significantly shaped the genomes of angiosperms during evolution. Two forms of polyploidy are often considered: allopolyploidy, which originates from interspecies hybrids, and autopolyploidy, which originates from intraspecies genome duplication events. Besides affecting genome organization, polyploidy generates other genetic effects. Synthetic allopolyploid plants exhibit considerable transcriptome alterations, part of which are likely caused by the reunion of previously diverged regulatory hierarchies. In contrast, autopolyploids have relatively uniform genomes, suggesting lower alteration of gene expression. To evaluate the impact of intraspecies genome duplication on the transcriptome, we generated a series of unique *Arabidopsis thaliana* autotetraploids by using different ecotypes. *A. thaliana* autotetraploids show transcriptome alterations that strongly depend on their parental genome composition and include changed expression of both new genes and gene groups previously described from allopolyploid *Arabidopsis*. Alterations in gene expression are stable, nonstochastic, developmentally specific, and associated with changes in DNA methylation. We propose that *Arabidopsis* possesses an inherent and heritable ability to sense and respond to elevated, yet balanced chromosome numbers. The impact of natural variation on alteration of autotetraploid gene expression stresses its potential importance in the evolution and breeding of plants.

allopolyploidy | autopolyploidy | evolution

Polyploidy has fundamentally influenced the speciation and evolution of plants and animals (1–6). To succeed, newly occurring polyploids must overcome notable challenges: genomic instability based on aberrant chromosome segregation during meiosis (3, 4, 6), and rapid adaption to selective environmental pressures that includes competition, for instance, with their diploid progenitors (4, 5, 7). Among known polyploid plants, allopolyploids show a taxonomic predominance (2, 3, 5). However, increasing evidence indicates that the actual appearance of autotetraploid plants in nature might be significantly underestimated (3, 5, 8, 9). The basis for their evolutionary success remains unclear.

Polyploidy has not only significantly shaped the genomes of plants throughout their evolutionary history (2, 9, 10) but has also impacted other genetic and epigenetic aspects including gene expression (4, 7). Studies on differential gene expression and transcriptomics have mainly focused on (neo-) allotetraploids such as wheat, cotton, maize (a segmental allotetraploid; ref. 11), and prominently, resynthesized *Arabidopsis suecica* from (neo-) tetraploid *A. thaliana* and *A. arenosa* (12–18). Transcriptional profiling of two *A. suecica* lines revealed that the expression of >1,400 genes diverged from the midparent value (16). This profiling demonstrated that allopolyploid plants exhibit considerable transcriptome alterations as compared with their diploid progenitors. As allopolyploids arise from interspecies hybrids, part of these changes are likely caused by reunion of previously diverged regulatory hierarchies. In contrast, autopolyploid plants, which result from intraspecies genome duplication, have uniform genomes whereby significant transcriptome alterations would be unexpected. Supporting this notion, an accompanying control experiment of the *A. suecica* analysis detected only negligible differences in gene expression between diploid and a tetraploid *A. thaliana* ecotype L*er* line (16). Similarly, the analysis of 9,000 genes in potato auto(poly)ploids revealed few very weak differences in comparison with diploids (19). Together with the uniformity of autopolyploid genomes, these albeit limited analyses suggested an absence of significant transcriptome alterations in autopolyploid plants, reminiscent to findings in tetraploid yeast (20).

We were interested to test whether significant gene expression alterations can be found among newly synthesized autopolyploids. A series of *A. thaliana* autotetraploids from nine different ecotypes was subjected to gene expression/transcriptome analysis. Our study uncovers an ecotype-dependent, heritable capacity to significantly change gene expression in autotetraploid *A. thaliana*.

Results

***A. thaliana* Col-0 but Not L*er*-0 Ecotype Shows Significant Transcriptome Alteration in Response to Tetraploidy.** To evaluate the impact of intraspecies genome duplication, we conducted a series of transcriptome analyses with numerous *A. thaliana* neo-autotetraploids (see Table S1 for overview of experimental layouts). First, we compared the seedling transcriptome of tetraploid Col-0 lines with their diploid Col-0 progenitor (Fig. 1*A*). Four recently generated independent tetraploid lines of the third generation, after induction, were used (21). Although tetraploid plants typically exhibited enlarged cells and tissues in comparison with diploids, overall structural morphology remained unchanged (Fig. 1*B*). These lines were repeatedly assessed by flow cytometry and chromosome counts for their ploidy (ref. 21; Fig. 1*C*). Transcriptome analysis defined 476 genes (286 up- and 190 down-regulated) that exhibited significant changes in gene expression (cutoff threshold, 1.5-fold; additional 112 genes displayed more subtle fold changes, FCs) (Dataset S1). We performed the same analysis with a series of independently generated tetraploid L*er*-0 lines (Fig. 1 *D* and *E*). In contrast to Col-0, comparison of tetraploid L*er*-0 vs. diploid L*er*-0 seedlings detected only nine genes of disparate functions (all >1.5-fold suppressed; Fig. 1*F*) (Dataset S2). Notably, these nine genes in L*er*-0 tetraploids were not altered between di- and tetraploid Col-0.

Using the same lines as described, the transcriptome of the sixth to eighth rosette leaves of tetraploid Col-0, versus diploid Col-0, was then analyzed to represent a second developmental stage and tissue. Correspondingly, 247 genes were differentially

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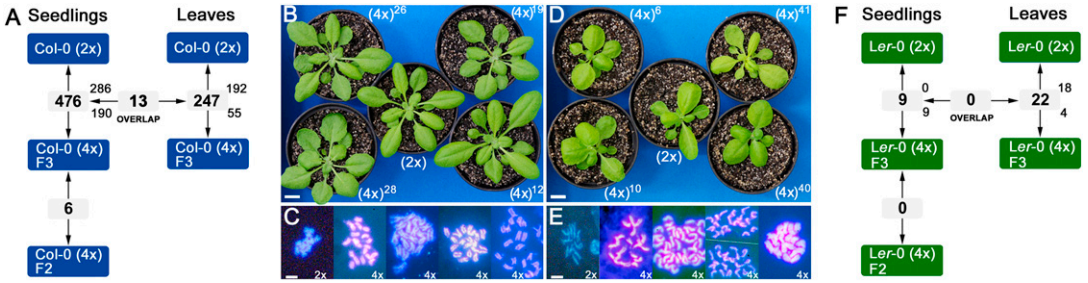

Fig. 1. Transcriptome alterations and morphology of autotetraploid *A. thaliana* Col-0 and L*er*-0. (*A*) Transcriptome alterations in Col-0 between diploids and tetraploids (tissues and generations as indicated). Up- or down-regulated genes at the top and bottom of the shaded boxes, respectively. (*B*) Morphology of diploid (2×) and tetraploid Col-0 (4×) lines (indicated by numbers) at the rosette stage. (*C*) Mitotic chromosome figures of Col-0 root tip cells (diploid line, tetraploid lines 12, 19, 26, and 28 from left to right). (*D*) Morphology of diploid (2×) and tetraploid L*er*-0 lines (indicated by numbers) at the rosette stage. (*E*) Mitotic chromosome figures of L*er*-0 root tip cells, at different mitotic stages (diploid line, tetraploid lines 6, 10, 40, and 41 from left to right). (*F*) Transcriptome alterations in L*er*-0 between diploids and tetraploids (tissues and generations as indicated; up- and down-regulated genes as in *A*). Only alterations with >1.5-fold changes are shown ($P < 0.05$). Note the morphological similarity of di- and tetraploid plants at the rosette stage. (Scale bars: *B* and *D*, 1 cm; *C* and *E*, 10 μm.)

expressed between the sixth and eighth tetraploid and diploid rosette leaves, of which 192 were more than 1.5× up- and 55 were more than 0.67× down-regulated, respectively (42 additional genes exhibited more subtle changes) (Dataset S1). Again, as observed in the seedlings, the sixth to eighth tetraploid L*er*-0 leaves exhibited few transcriptome changes; in total 22, with 18 up- and 4 down-regulated (five additional genes exhibited more subtle changes) (Dataset S2). Although the microarrays used are based on the Col-0 sequence (22), we calculated that this explains little of the response difference of tetraploid L*er*-0 vs. Col-0 (*SI Materials and Methods*). Thus, upon shift from di- to tetraploidy, Col-0 responds with the alteration of gene expression of several hundred genes, whereas L*er*-0 shows minimal altered gene expression. We classified these ecotypes as responder (Col-0) and nonresponder (L*er*-0), respectively.

Alteration of Gene Expression Response to Tetraploidy Depends on Developmental Stage. The Gene Ontology (GO) groups represented by the detected genes, as described in The Arabidopsis Information Resource (TAIR) representations, covered almost all important functional groups of biological processes and molecular functions (Fig. S1). Further, an analysis for significant enrichments of GO groups refined this overview and uncovered under- and over-representation related to various functions/processes (Dataset S3). This analysis was extended by a deeper term-supported comparative in silico analysis based on term-supported matching (*Materials and Methods*), which delivered a striking enrichment of genes related to specific functional categories (Fig. 2*A*). In seedlings, we found gene groups related to photosynthesis and chlorophyll, sugar and cell wall biosynthesis, metal ions, calcium, ATPases, and transcriptional control including six NAC transcription factors (Fig. 2*A*). Several of the most highly up- or down-regulated genes covered ethylene-, stress-, senescence- and defense-related processes, respectively, many with adjusted *P* values far below 0.05 (Table S2). Subsequent RT-PCR tests on diploid and tetraploid tissue directly compared amplification products on gels. Only those genes that showed clear differences were further followed. According to this preselection, ≈55% of the selected genes (Figs. 2*B* and 3; together for seedling and leaf material) displayed significant differences between di- and tetraploid gene expression. These cases enabled us to control the representation of alterations in gene expression in the different functional categories by quantitative RT-PCR (qRT-PCR) (Fig. 2*B*). In comparison with the seedlings, the in silico scan of the detected Col-0 leaf genes indicated a significantly changed pattern of altered activity (Fig. 2*A*). The "cell wall/sugar program" had been extensively reduced, 87 vs. 26 genes, with only two overlaps (*At1g22400* and *At4g30270*). Only one NAC transcription factor was found. The seedling ethylene/stress program had been considerably reduced in "favour" of an auxin synthesis/signaling program with many IAA-antagonists of auxin responsive factors (ARFs), and Short Auxin Upregulated RNAs (SAUR)-like genes (20 genes; FCs > 2.0; Table S3). Microarray data were confirmed by qRT-PCR analyses of genes representing diverse GO functional groups (Fig. 2*B*). One particularly interesting case included an overexpressed SAUR gene-cluster (designated *At5g180-c*) comprising six highly homologous copies (Fig. S2). The genes are dispersed in a region of 20 kb with some copies <2 kb apart. Overexpression of *At5g180-c* was predominantly caused by *At5g18010*. Its overrepresentation within cDNA clones was 12/58 in tetraploids vs. 2/29 in diploids. Only 13 of the genes with >1.5-fold up- or down-regulation, respectively, overlapped between seedlings and leaves (Fig. 1).

Transcriptome of Tetraploid Col-0 and L*er*-0 Is Highly Stable in Consecutive Generations. The tetraploid L*er*-0 and Col-0 lines analyzed in this study exhibited high chromosome number stability during consecutive generations (21). We investigated the stability of the tetraploid transcriptome by analyzing microarray expression profiles of seedlings, two and three generations after induction. This analysis revealed an almost complete identity at a genome-wide level. We did not find any differences in the second vs. third tetraploid L*er*-0 comparison. The comparison of second vs. third tetraploid Col-0 revealed only six differences (Fig. 1 and Dataset S1). Thus, both the unaltered and the altered tetraploid transcriptome of the nonresponding L*er*-0 and the responding Col-0, respectively, remain genetically stable.

Microarray Analysis Detects a Species-Specific Locus That Is Strongly Overexpressed in Both Seedlings and Leaves. Among the transcripts more abundant in tetraploids than in diploids was *At1g53480*. The corresponding gene, named *MRD1,* had been shown to be transcriptionally suppressed in a former microarray analysis of the *Arabidopsis mto1-1* mutant (23). However, its function remained unclear. *MRD1* is (weakly) expressed throughout the adult plant development i.e., in seedlings, young rosette leaves, old rosettes, and siliques (ref. 23; Fig. 3 *A* and *B*). Analysis of T-DNA insertion lines (Fig. 3*C* and *SI Materials and Methods*) did not reveal a conspicuous phenotype with respect to seedling viability, overall morphology, and fertility. *MRD1* and its homolog *At5g03090* appear to be species-specific loci of unknown function, because truncated copies were only found in *A. lyrata* among all plant sequence compilations (Fig. S3). *MRD1* displays a weak basic expression in diploid Col-0, diploid L*er*-0, and tetraploid L*er*-0. However, as verified by qRT-PCR, this locus displayed >20- to 110-fold (leaves vs. seedlings) overexpression in tetraploid Col-0 (Fig. 3*A*; compare also with Fig. 4). Northern blot analysis of *MRD1* confirmed this observation (Fig. 3*B*). Interestingly, *MRD1* overlaps with a second gene coded on the opposite strand, *At1g53490*. This gene is also altered in its expression in tetraploids but at much lower level. Although overlapping, this gene seems

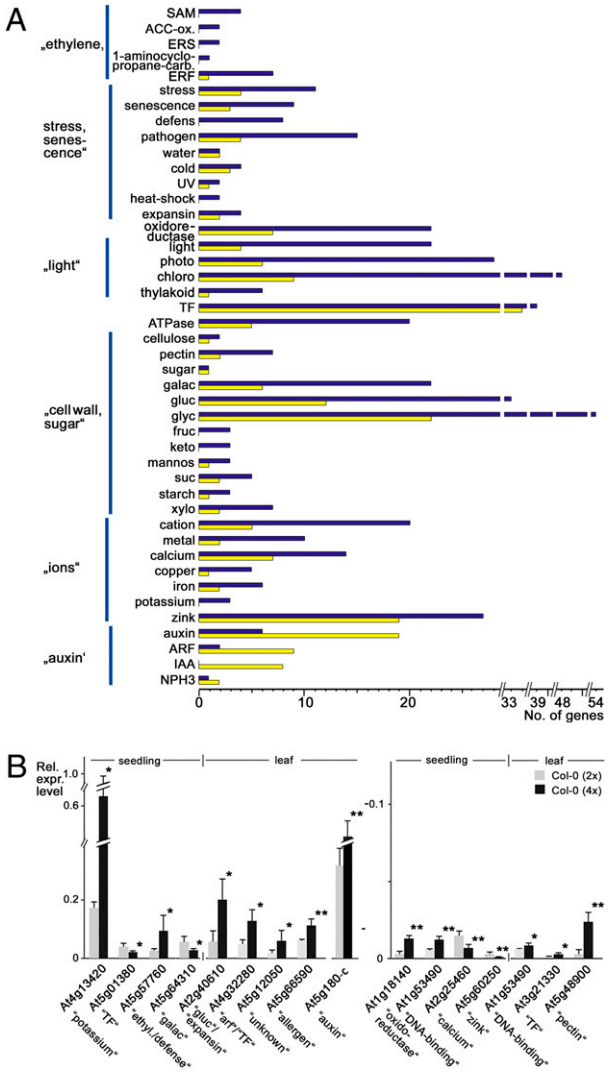

Fig. 2. Development and transcriptome alteration in tetraploid Col-0. (*A*) Comparison of conspicuous functional GO terms with altered expression in Col-0 tetraploids: seedling (blue bars) vs. leaf (yellow bars). Terms in quotation marks indicate key processes covered by the selected functional terms (for details see text and *SI Materials and Methods*). (*B*) Altered expression of selected genes in *A. thaliana* Col-0 autotetraploids as shown by qRT-PCR of genes representing disparate functional categories. At5g180-c indicates qRT-PCR of a complete "SAUR-like" gene cluster comprising six members: *At5g18010-30*, *At5g18050-60* and *At5g18080*. The reference gene in these analyses was *ACT2* (as in ref. 16). This analysis verified 55% of the selected genes from the microarray analysis to be altered between di- and tetraploids. Significance values of one-tailed *t* test: $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.0005$; bars with SD.

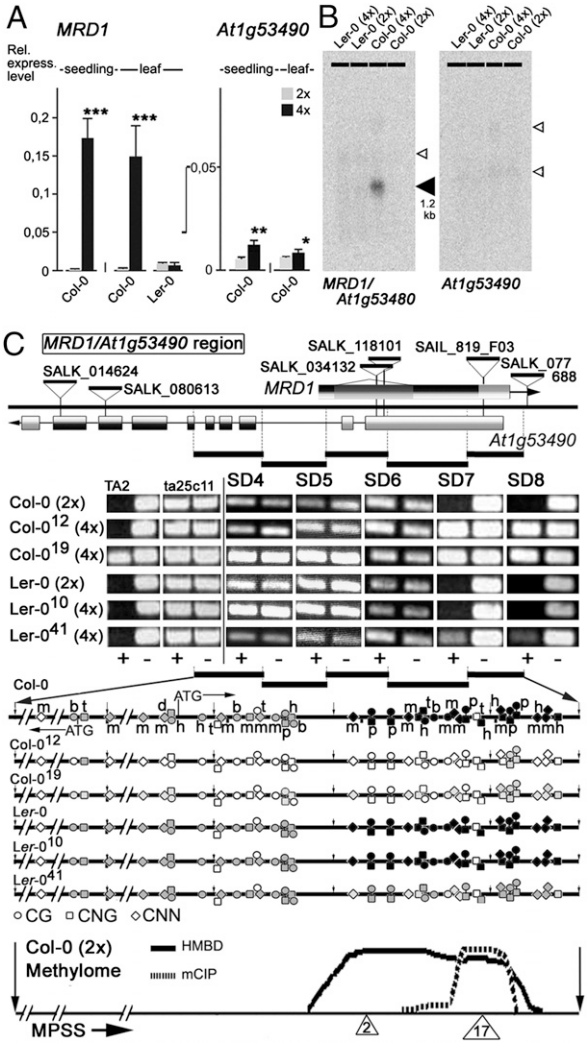

Fig. 3. Expression and methylation of *MRD1*(*At1g53480*) and *At1g53490*. (*A*) qRT-PCR of *MRD1* and *At1g53490* in diploid vs. tetraploid Col-0 and Ler-0. (*B*) Northern blot with *MRD1* (*Left*) and *At1g53490* (*Right*). Size of approximate *MRD1* transcript length is given (filled arrowhead); open arrowheads indicate weak bands probably including homologous gene copies. (*C*) Integrates analysis with methylation requiring (McrBC) and methylation sensitive enzymes. (*Top*) Structure of the *MRD1*/*At1g53490* region including tested T-DNA insertions. A TAIR annotated intron (triangle) was not found in this study. (*Middle*) McrBC analysis of subregions SD4-SD8, transposon TA2, and an nonmethylated ta25c11 repeat DNA sequence tile (taken from ref. 33). Complete methylation is indicated by the absence of a band ("+" and "–" indicate that McrBC was included or excluded, respectively). Note the demethylation of TA2 in Col-0^{19} (4×). (*Bottom*) Methylation and demethylation at sites for BstUI (b), MboI (m), DrdI (d), HpaII/MspI (p), Hpy188III (h), and TseI (t) resolved as CG, CNG, and CNN methylation sites is indicated by circles, squares, and diamonds, respectively. Shading indicates strong (black), weak (dark and light gray) and no methylation (blank). The methylome in this region (24) shows strong methylation at the 3′-end of *MRD1* for Col-0 (2×) detected with monoclonal methylcytosine antibodies (mCIP) and affinity purification with the methylcytosine binding domain of human MeCP2 (HMBD) (24). The MPSS project (26) revealed several short RNA signatures in particular for the 3′-region of *MRD1* (numbers in triangles). Lines and ploidies are indicated. For details, see text, *SI Materials and Methods*, and Figs. S3 and S4.

not to detectably affect the expression of *MRD1* (Fig. 3 *A* and *B*). This finding is in line with other pairs of overlapping genes (24). Alteration of (trans) gene expression has been shown to correlate with epigenetic phenomena in tetraploids, including modulation of DNA methylation (12, 15, 25). We therefore performed DNA methylation analyses, which scanned the methylation status of consecutive segments of this region by comparing the effects of methylation-sensitive enzymes with the enzyme McrBC, which cuts only when DNA contains methylated cytosines (Fig. 3*C* and Figs. S3 and S4). This analysis showed that low transcriptional activity of *MRD1* in tetraploid L*er*-0 is accompanied by partial or complete methylation in the 3′-region, whereas in tetraploid Col-0, its strong expression is correlated with strong demethylation in the same region (Fig. 3*C*). The promoter region of *MRD1* is generally, although not completely, demethylated in di- and tetraploid lines. In fact, the methylome project of diploid Col-0 has shown that this gene is "body-methylated" not "promoter-methylated" (ref. 24; TAIR9 GBRowse: http://gbrowse.arabidopsis.org/cgi-bin/gbrowse/arabidopsis/). In addition, data from the Massively Parallel Signature Sequencing (MPSS) project indicate an accumulation of small

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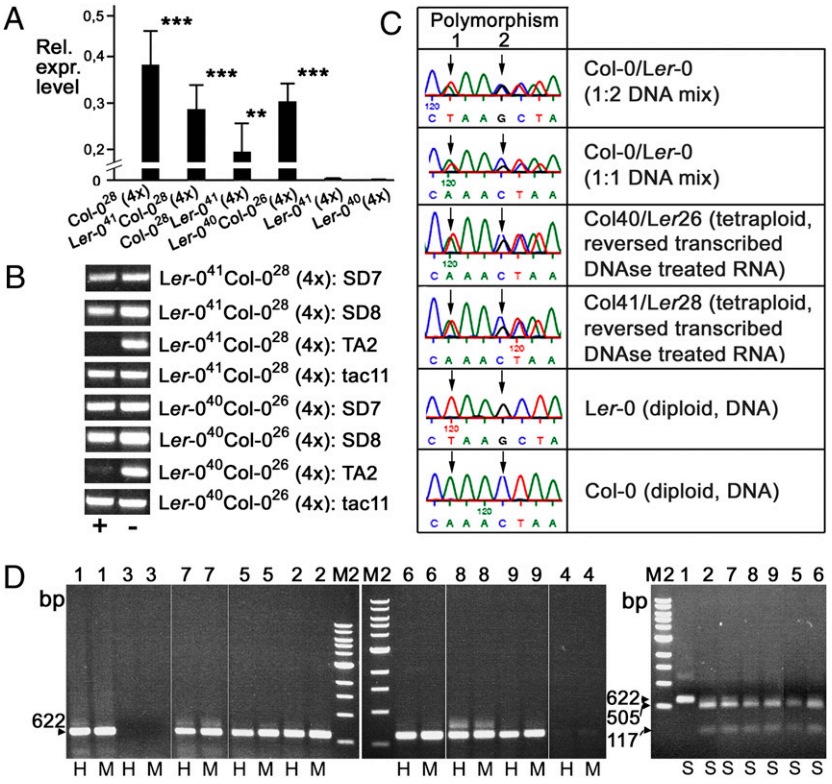

Fig. 4. Inheritance of *MRD1* overexpression and methylation in tetraploid *A. thaliana* F_1 hybrids. (*A*) Real-time qRT-PCR of leaf material of hybrid tetraploid Col-0/L*er*-0 combinations. (*B*) McrBC-Methylation analysis of *MRD1* (regions SD7 and SD8; Fig. S3). Comparisons with TA2 and the ta25c11 (=tac11) are as in Fig. 3. (*C*) Sequence reactions of RT-PCR-amplified transcripts identifying $MRD1^{Col}$ and $MRD1^{Ler}$ alleles in tetraploid Col-0/L*er*-0-hybrids. Arrows point to positions of sequence polymorphisms in *MRD1* (Fig. S3). (*D*) Genomic DNA of diploid lines Col-0 [1] and L*er*-0 [2], tetraploid lines Col-0^{12} [3], Col-0^{19} [4], L*er*-0^{10} [5], L*er*-0^{41} [6], tetraploid hybrids L*er*-0^{40}/Col-0^{26} [7], L*er*-0^{41}/Col-0^{28} [8], and Col-0^{28}/L*er*-0^{41} [9], respectively, were digested with HpaII (H) and MspI (M) (left gels). PCR was performed by using primers flanking a Col-0/L*er*-0 StuI restriction enzyme polymorphism (Fig. S3). Blocking of HpaII/MspI digestion enabled the generation of a band (at 622 bp). The resulting bands amplified from the HpaII and MspI-digested genomic DNA were isolated, purified, combined for each line, redigested with StuI (*S*), and separated again (right gel). Significance values of one-tailed *t* test: $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.0005$; bars with SD (comparison with tetraploid L*er*-0^{40} and L*er*-0^{41}). Lines are as in Fig.1, with ecotypes and ploidies indicated.

RNAs in particular for the 3′-region of the gene (Fig. 3*C* and ref. 26; TAIR9 GBrowse).

Altered Transcription of *MRD1* in Tetraploids Is Heritable. The striking difference between Col-0 and L*er*-0 neo-tetraploids allowed us to test for *MDR1* expression in tetraploid hybrids and, consequently, for inheritance and outcrossing of the tetraploid *MDR1* Col-0 response. We therefore generated reciprocal tetraploid Col-0/L*er*-0 hybrids by using the established lines. In fact, qRT-PCR showed that the capability of sensing and responding to tetraploidy by Col-0 is transmitted to the hybrid (Fig. 4*A*). In addition, methylation analyses showed that this overexpression was accompanied by maintained demethylation at this locus (Fig. 4*B*). Because $MRD1^{Col\text{-}0}$ and $MRD1^{Ler\text{-}0}$ display several polymorphisms (Fig. S3) we directly sequenced reverse transcribed mRNA from different tetraploid Col-0/L*er*-0 hybrids to assess ecotype specific polymorphisms in the transcripts. Interestingly, the sequence signal peaks indicated that both Col-0 and L*er*-0 *MDR1* alleles were expressed with the same intensity (Fig. 4*C*). Thus, the transcription of $MRD1^{Ler\text{-}0}$ appeared to be higher in F_1 Col-0/L*er*-0-hybrid tetraploids than in diploid and tetraploid L*er*-0. We then analyzed the methylation status of *MRD1* in the hybrids by taking advantage of a polymorphic StuI-restriction enzyme recognition site (present in L*er*-0 and absent in Col-0 SD7 region; Fig. S3). Purified SD7-DNA, which blocked methylation-sensitive HpaII and MspI enzymes turned out to originate almost exclusively from L*er*-0 (Fig. 4*D*).

***A. thaliana* Tetraploid Transcriptome Response Is Ecotype Specific.** We were interested to test whether some of the genes detected, in particular *MRD1*, would show expression alteration response in other ecotypes. We generated tetraploids of seven additional ecotypes. Because of its strong overexpression, we reasoned that *MRD1* might be a valuable tool for monitoring ploidy-affected gene expression in *A. thaliana*. In fact, expression analyses of leaf material of the new neo-tetraploid ecotypes revealed considerable variability with respect to absolute and relative expression differences of *MRD1* (Fig. 5*A*). Not surprising, the absolute expression levels differed between ecotypes. For four of seven ecotypes significantly altered *MRD1* expression was observed. This test was complemented with experiments by using two additional genes, *IAA29* (*At4g32280*) and the SAUR-gene cluster (*At5g180-c*). These experiments uncovered almost the same variability (Fig. 5 *B* and *C*). CT-1 and L*er*-1 turned out to be nonresponders in all cases, whereas the other five showed significant expression differences in two or all three genes analyzed.

Discussion

Alteration of Transcriptome in *A. thaliana* Autotetraploids Depends on Ecotype, i.e., Genome Composition. It was generally expected that the uniform genomes of autopolyploids, in contrast to those of allopolyploids, should not exhibit significant gene expression alterations. This observation is supported by limited analysis (16, 19). The presented data on Col-0 vs. L*er*-0 transcriptome comparison demonstrate significant ecotype specific differences in gene expression alterations when the diploid is compared with the tetraploid. Col-0 alters several hundred genes in two tissues, suggesting that more might be uncovered in other tissues. Although this amount is significantly less than found in allote-

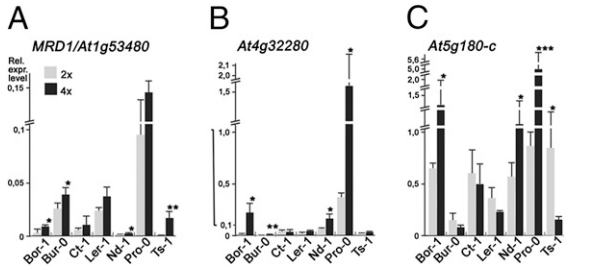

Fig. 5. Di- vs. tetraploid expression profile of selected genes in various *A. thaliana* ecotypes. (*A*) Expression of *MRD1* in seven additional diploid vs. tetraploid ecotypes (qRT-PCR). (*B*) Same analysis as in *A* for *IAA29/At4g32280*. (*C*) Same analysis as in *A* for SAUR gene cluster *At5g180-c*. Leaf material, ecotypes, and ploidies are indicated. Significance values of one-tailed *t* test: $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.0005$; bars with SD. For details, see text.

traploids (16), it is in sharp contrast to tetraploid L*er*-0, which displays an almost diploid expression profile. Limited analysis of other ecotypes with selected probes supports the notion that the response to tetraploidy is variable and depends on the genomic composition. In the Bor-1 and Nd-1 ecotypes, all three genes were up-regulated, whereas in other ecotypes, only two of the genes were altered in their expression. Ct-1 and L*er*-1 did not show any response to all three genes. Whether this observation indicates variable degrees of response capability has to be further investigated. Thus, in answer to our question, some autopolyploids react in a similar, but more subtle way than allopolyploids. It should be mentioned, that the ecotype specific gene expression alterations shown in this study are also clearly distinct from aneuploid syndromes (27–29), because they occurred in *A. thaliana* autotetraploids, i.e., balanced euploids. In contrast, aneuploidy is an out-of-balance situation leading to extensive gene expression alterations in *Arabidopsis* (28) and segregation distortion of loci such as *Arabidopsis SENSITIVE TO DOSAGE IMBALANCE* (*SDI*) (29).

Transcriptome Alterations in Autotetraploid *Arabidopsis* Are Developmentally Specific. The data show that gene expression alterations in autotetraploids are developmental stage specific. This finding is reflected by the low overlap (13 genes) between altered seedling and leaf transcriptomes and by different representation of GO groups (Fig. 1 and Datasets S1 and S3). Apparently, the Col-0 response is a general alteration or relaxation of gene expression control covering genes of different stages. The functional gene groups displayed by seedlings and leaf are well known from these tissues. Seedlings display a biphasic mode of ethylene-related gene activity (30), whereas any form of leaf organogenesis is tightly linked to localized auxin accumulation and auxin-driven gene activities (31, 32). Interestingly, neo-allopolyploid *A. suecica* also revealed a conspicuous alteration of ethylene/stress-related genes (16) showing partly similar reactions in both forms of polyploidy. However, they also revealed different gene expression alterations not observed in autopolyploids such as those considering heat shock genes. It is likely that some of these genes are active or inactive during stages, which do not correspond to the developmental program of their parents.

***A. thaliana* Transcriptome Alteration Response to Tetraploidy Has a Genetic Basis and Displays Epigenetic Phenomena.** The comparison of Col-0 vs. L*er*-0 tetraploids clearly showed that the transcriptome alteration response does not depend on the chromosome number per se, but on the origin of the chromosomes. Furthermore, the alteration was completely transmitted through selfing to the next generation. Selecting a strongly overexpressed gene (*MRD1/At1g53480*) to study the transmission in reciprocal crosses of Col-0 × L*er*-0 tetraploids demonstrated that the response in Col-0 is transmitted to the hybrids as well. Notably, in these cases, only two chromosome sets originate from the "responsive" Col-0 ecotype. Taken together, this result suggests that Col-0 but not L*er*-0 possesses one or more genetic factors that are capable of sensing the alteration of genome dosage and inducing gene expression alterations. Also, the analysis of other ecotypes shows that this ability depends at least partly on the genotype. Possibly, the absence of *MRD1* overexpression in some tetraploids is due to mutation. It is known that L*er*-0 originates from X-irradiated parents (NW20; TAIR). However, the reasons for the observed expression alterations might be more complex. For instance, diploid Col-0 and L*er*-0 genomes possess variable DNA methylation patterns (33). Although this natural epigenetic variability seems not to cause significant gene expression differences in diploids (33), we do not know whether this variability could contribute as such at the tetraploid level.

At this point of discussion, it seems necessary to differentiate between sensing vs. induction vs. transmission/preservation. Although we do not know the sensing factors, we can speculate what they could sense. Altered nuclear surface to volume ratios in tetraploids have been discussed as causative for gene expression/regulatory changes (4, 34). Polyploids generally show increased nuclei, which implies an altered nuclear surface to volume ratio. The gene expression alteration of *MRD1* in various Col-0 vs. L*er*-0 tetraploids and Col-0/L*er*-0 tetraploid hybrids are strongly correlated with DNA (de)methylation. Several analyses of selected (trans) genes have demonstrated changes in gene expression between plants with altered ploidy grade (12–15, 25, 35, 36), some of these have also uncovered a link to epigenetic phenomena, in particular DNA (de)methylation. Upon sensing a higher chromosome number in a nucleus with an altered surface to volume ratio, the induction of DNA (de)methylation of selected genes could be caused by targeted re- and demethylation mechanisms, which have been recently discovered in *Arabidopsis* (37, 38). These and similar mechanisms are also responsible for the preservation of the DNA methylation. Basically, the study of *MRD1*, which belongs to the ≈33% "body-methylated" *A. thaliana* genes (24), indicates one epigenetic option for maintaining the observed transcriptome alterations. However, the observed alterations should not be assigned to DNA methylation alone. Epigenetic effects can be based on other DNA modifications. Furthermore, alteration of the DNA methylation pattern of one transcription factor/repressor could be sufficient to alter the expression of other genes without any further change of their methylation.

Based on the sequence data of reversed transcribed *MRD1*-RNA, it is tempting to speculate that $MRD1^{Ler\text{-}0}$ displays higher transcriptional activation in the hybrids. This activation could happen post fertilization unlike transcriptional reactivation of transposons in pollen (39). Alternatively, this gene could be activated during gametogenesis and then silenced upon fertilization. Then this silencing would be suppressed in tetraploid Col-0 and F_1 Col-0/L*er*-0 hybrids because of the presence of chromosomes originating from tetraploid Col-0. The final effect resembles the opposite of paramutation of loci such as maize B-I (40). However, it is also possible that the dosage of a suppressor not present in Col-0 is diluted in the hybrids. This observation is also complicated by the fact that a considerable part of $MRD1^{Ler\text{-}0}$ is strongly methylated in the hybrids. In addition, there is always a basal level of *MRD1* transcription in the tissues tested regardless of the ploidy level. The data of the MPSS project (26) suggest that methylation and, in turn, activity of *MDR1*, could be influenced by small iRNA-linked mechanisms. In this context, it is worth it to mention that *MRD1* was found to be suppressed in the *mto1-1* mutant, which overaccumulates soluble methionine (23). Taken together, our observations indicate a complex control of *MRD1* transcription and it remains to be determined whether paramutation-like phenomena are involved.

Implications for Evolution and Plant Breeding. Significant changes in cellular morphology and physiology are known in allo- and autotetraploids (1–5, 15–18, 21). In the former, some trait changes have clearly been associated with gene expression alterations (17, 18). Similar effects are expected to occur in *Arabidopsis* autotetraploids. Here, we consider solely the potential of such

alterations in context of the evolution of these two forms of ploidy. The data on allotetraploids together with our observations open up alternative evolutionary scenarios for allo- vs. autopolyploids, which both exhibit equally stable chromosome segregation (3, 6). Allopolyploids and their homoploid progenitors could resort to numerous alterations in gene expression, allowing for rapid adaptations to extreme habitats. On the other hand, they might be prone to developmental accidents due to the interference of ploidy, heterosis, and effects that result from the reunion of divergent genomes (2, 4, 6, 7, 9, 41, 42). Neo-autopolyploids could resort to a lower and stably heritable number of ploidy-induced alterations allowing selective adaptations. In the long term, these processes might entail mutations that would act to fix such alterations (7), which would otherwise be lost. If so, this mechanism could appreciably impact the evolution of autopolyploids, together with other known mechanisms such as point mutations or genetic drift. Additional aspects complicate these considerations. First, autopolyploidy can occur recurrently (5, 10, 42). Second, autopolyploids could "feed" allopolyploid evolution. For instance, the generation of synthetic *A. suecica* allopolyploids was only possible through crosses of synthetic autotetraploid *A. thaliana* with *A. arenosa* because of the lethality of homoploid hybrids (15). Allopolyploids are taxonomically predominate, but a reliable estimate for the frequency of autopolyploid species is yet to be found. In fact, autopolyploids might be much more prevalent in nature than presently known (2, 3, 5, 6, 8, 9, 41), because they are sometimes difficult to recognize based on morphology. Our results support this notion and indicate that the success of autotetraploids might critically depend on the magnitude of a species' natural genetic variability. This observation could impact plant breeding because autopolyploidy might be much better exploited if the natural variability of a species is considered.

Materials and Methods

Plant Material. European Arabidopsis Stock Centre (Loughborough, UK) and *Arabidopsis* Biological Resource Center (Columbus, OH) provided *A. thaliana* ecotypes. We used established (21) or converted new ecotypes to tetraploids as described (21).

Gene Expression and Microarray Analysis. Protocols for isolation, purification, and storage of (c)RNA, (q)RT-PCR analysis, and (q)RT-PCR-primers can be found in *SI Materials and Methods* and Table S4. The *Arabidopsis* 60-mer OligoMicroarray Agilent 4 × 44K platform was used. Cy3/Cy5-two-color experiments comprised at least four biological replicates (Table S1). Seedling transcriptome analyses between diploid L*er*-0 vs. Col-0 and between tetraploid Col-0 vs. L*er*-0 lines revealed 860 and 348 ecotype specific differences, respectively. These and the other microarray data in this work are deposited at NCBI/GEO; accession no.: GSE18482.

Additional Experimental Procedures. A detailed description of experimental procedures including microarray, methylation, and qRT-PCR analysis as well as bioinformatics and statistics can be found in *SI Materials and Methods*.

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