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Genetics

A change of ploidy can modify epigenetic silencing

(*Arabidopsis thaliana*/epigenetic regulation/gene silencing/polyploidy/polysomaty)

ORTRUN MITTELSTEN SCHEID*†, LUDMILA JAKOVLEVA*‡, KARIN AFSAR*, JOLANTA MALUSZYNSKA§, AND JERZY PASZKOWSKI*

*Friedrich Miescher Institute, P.O. Box 2543, CH 4002 Basel, Switzerland; and §Department of Plant Anatomy and Cytology, Silesian University, Jagiellonska 28, 40-032 Katowice, Poland

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ABSTRACT **A silent transgene in *Arabidopsis thaliana* was reactivated in an outcross but not upon selfing of hemizygous plants. This result could only be explained by assuming a genetic difference between the transgene-free gametes of the wild-type and hemizygous transgenic plants, respectively, and led to the discovery of ploidy differences between the parental plants. To investigate whether a change of ploidy by itself can indeed influence gene expression, we performed crosses of diploid or tetraploid plants with a strain containing a single copy of a transgenic resistance gene in an active state. We observed reduced gene expression of the transgene in triploid compared with diploid hybrids. This led to loss of the resistant phenotype at various stages of seedling development in part of the population. The gene inactivation was reversible. Thus, an increased number of chromosomes can result in a new type of epigenetic gene inactivation, creating differences in gene expression patterns. We discuss the possible impact of this finding for genetic diploidization in the light of widespread, naturally occurring polyploidy and polysomaty in plants.**

Gene expression levels are influenced by at least three classes of parameters: (*i*) genetic components (cis-elements of the genes themselves) that determine the potential interaction with transcription factors are inscribed in the nucleotide sequence of the gene and are changed only by rare, essentially irreversible mutations; (*ii*) regulatory factors act in trans in response to developmental or environmental stimuli, allowing rapid yet reversible adaptations which may readily be altered in progeny cells; (*iii*) there can be alterations in gene expression that are either programmed, induced, or apparently spontaneous, and are maintained with surprising stability in progeny of the affected cell. The frequency of such changes (and their reversion) exceeds that of mutation, yet they are transmitted from one cell to its progeny in a mode reminiscent of genetic changes. Therefore, this type of control is described as "epigenetic" gene regulation, a term originally created to describe stable changes of gene expression in somatic cells.

An epigenetic change may affect many genes simultaneously or only a specific gene. An example of extensive and programmed epigenetic, developmentally triggered regulation is the gene dosage compensation achieved by the inactivation of genes located on "supernumerary" X chromosomes in female mammals, reducing the level of X-encoded gene products to that in cells with the single X male karyotype (1). An example of epigenetic regulation of an individual gene is the phenomenon of paramutation in which an interaction between alleles leads to the directed heritable change of expression for one allele (2, 3). If a paramutagenic allele is combined with a susceptible, paramutable allele, gene expression from the latter is reduced, either irreversibly or reverting slowly only after genetic segregation of the alleles.

Loss of gene expression by epigenetic change only comes to our attention if the gene product is not essential for the survival of the cell. X chromosome inactivation always spares one allele of the X-located genes on the active chromosome, and paramutation has been described mainly for genes involved in plant pigmentation where variegation is not detrimental in most cases and easily detectable. Presumably other genes can be regulated in a similar, epigenetic way, and since it is possible to integrate additional, well-characterized transgenes into the genomes of animals and plants, this question can be addressed by scoring for the expression of foreign gene products that are functional but not essential for the host organism.

The reduction or loss of expression of previously active transgenes has been described repeatedly and is referred to as epigenetic gene silencing (4–6). It is frequently provoked by specific sequence duplications, which is reflected in the term "homology-dependent gene silencing" (7, 8).

In the course of our work with transgenic *Arabidopsis* plants carrying a selectable marker gene, we have observed a surprising example of epigenetic inactivation without the involvement of homologous loci. We describe the phenomenon here and suggest a regulatory involvement of chromosome number. We further discuss possible implications for autosomal gene dosage compensation in the development of polysomatic plants and for the successful establishment of polyploid plant varieties.

MATERIALS AND METHODS

Strains of *Arabidopsis thaliana* ecotype Zürich transgenic for the hygromycin phosphotransferase gene (*hpt*) fused to the 35S promoter of cauliflower mosaic virus were obtained by PEG-mediated direct gene transfer to mesophyll protoplasts (9) and have been described in detail (10).

All individual parental plants involved in the crosses described in this paper were analyzed by Southern blot or PCR analyses and by callus or seedling resistance assays of their selfed progeny to confirm genotype and phenotype, respectively. Conditions for DNA and RNA isolation and Southern and Northern blot analysis have been described (10). Hybridization signals from the transcript on the Northern blots were quantified with a PhosphorImager (Molecular Dynamics) by using rectangular, equally sized areas surrounding the full-length transcripts in each lane. PCR analysis was performed on alkali-denatured leaf tissue (11) with *Taq* polymerase (Boehringer Mannheim) during 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with 50 nM each of primers amplifying part of the *hpt* sequence [5′-GTCCTGCGGGTAAATAGCTGCGCC and 5′-GTGTATTGACCGATTCCTTGCGG (12)]. Plants used for crosses were grown in soil in a controlled environment (70–80% relative humidity, 12 h light at 20°C/12 h darkness at 16°C). Flower buds (three to six per shoot) were

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†To whom reprint requests should be addressed.
‡Present address: Timiryazev Institute of Plant Physiology, Botanicheskaya 35, 127276 Moscow, Russia.

emasculated by removing the immature anthers with sterilized forceps. Cross-pollination was performed 2–3 days after emasculation. Siliques were harvested 3 weeks later, and mature seeds were kept for at least 1 week at 4°C to break dormancy. After surface sterilization (5 min in 5% calcium hypochlorite with 0.1% Tween 80, followed by extensive washing in sterile distilled water), seeds were allowed to germinate in axenic culture conditions on germination medium (13) in the presence or absence of 15 mg of hygromycin B per liter (Calbiochem). Germination rates were similar under selective and nonselective conditions. The number of viable seedlings was scored twice after 2 and 3 weeks. The percentage of hygromycin-sensitive seedlings was calculated from the number of nonviable seedlings appearing on selection medium, after subtracting the number of nonviable seedlings found without selection. Statistical analysis was performed according to ref. 14. Conditions for callus induction from tissue explants were described previously (10). Chromosome numbers were analyzed in 4′,6′-diamidino-2-phenylindole (DAPI)-stained squashes of the main root meristem (15). Tetraploid, nontransgenic plants were obtained among progeny of wild-type plants after repeated application of colchicine solution (0.1%) to the flower meristems (16).

RESULTS

We have previously described several transgenic lines of *A. thaliana* in which the transgene underwent epigenetic silencing. The lines used in the experiments described here are C1 and A (10). The transgenic insert of line C1 consists of a single copy of the *hpt* gene. This gene is inherited in the active state as a dominant Mendelian trait conferring hygromycin resistance over several generations. In contrast, line A contains multiple, rearranged copies of the *hpt* gene at a single genetic locus. The R0 generation of line A was clearly hygromycin resistant, but the selfed progeny were sensitive to the antibiotic in spite of the presence of the transgenic insert. The transgene was transmitted in an inactive state to subsequent generations obtained by self pollination, but surprisingly, outcrosses of sensitive S1 plants of line A to the nontransgenic wild type produced 6% progeny resistant to hygromycin, in both orientations of the cross (10). The occurrence of resistant plants in the outcross to the nontransgenic wild type, but not among the selfed progeny, was difficult to explain. The parental plants of line A were definitely hemizygous for the silent transgenic locus since the selfed progeny segregated 3:1 for seedlings containing and lacking the transgenic locus, respectively. Consequently, their selfed progeny are expected to consist of 50% hemizygous individuals. Outcrosses of hemizygous A plants should also result in 50% hemizygous progeny plants. Because half of the progeny is hemizygous in both cases, it is rather surprising that the reactivation was observed only after outcrossing. Therefore, we concluded that the gametes contributed by the nontransformed wild type in the cross had a different influence on transgene activity from gametes which were also free of transgene but which originated from the hemizygous transgenic plant in the course of self fertilization.

The presence of resistant individuals among the outcross progeny can be considered in two ways: either the outcross consolidates a configuration under which gene activity is maintained, or the outcross abolishes conditions under which gene activity is suppressed. In other words, there is either an activating influence of the wild-type gamete during outcrossing or a suppressing effect by the "empty" gamete of the hemizygous transformant during selfing. Gene reactivation was observed for only 6% of hybrid seedlings, whereas gene suppression seemed to affect 100% of the hemizygous selfed plants. We therefore decided to examine by test crosses whether the nontransgenic gametes from line A exhibit any suppressive effect on the active transgene of line C1.

We performed Southern blot analysis with DNA from progeny plants of several individuals derived from a hemizygous plant of line A by selfing, using the complete transformation plasmid as a probe. We identified plants that contained (A^+) and lacked (A^o) the transgenic locus. No signal was detectable in Southern blots with A^o DNA even when the blots were washed under conditions of low stringency (4× standard saline citrate, 50°C; data not shown).

We then addressed the question whether the transgene-free genotype A^o was able to suppress the activity of the *hpt* transgene. The partners for these test crosses were homozygous plants of line C1; thus every progeny plant was expected to inherit one active allele from this parent and should be hygromycin resistant. This expectation was met in the cross of C1 with the nontransformed wild type: crosses in both directions yielded close to 100% resistant progeny (Table 1). In contrast, crosses of C1 with the line A^+ produced 5.6% sensitive plantlets (Table 1). This result could have been interpreted as silencing of the C1 locus mediated by the homologous, inactive transgene of A^+, but to our surprise the crosses between C1 and line A^o resulted in even more (11.9%) sensitive seedlings (Table 1). Application of the χ^2 test indicated that the number of viable seedlings was only significantly different under selective conditions. Thus, a subpopulation of the hybrid seeds had lost the hygromycin resistance after the cross of C1 to an A-derived parent, regardless of whether this contained the transgene (A^+) or not (A^o). Since A^o did not contain any DNA homologous to the transgenic locus C1, it can be concluded that this process does not require the presence of a homologous silencing locus and, therefore, must be due to other genetic determinants.

A clue to one possible difference between the nontransformed wild type and line A^o lacking the transgene came from another peculiarity of line A. When screening the S2 population of line A for an individual homozygous for the silent insert, which required Southern analysis of individual progeny

Table 1. Suppression of an active transgene by lines A^+ and A^o

Cross*	Selection	Seedlings tested, no.	Nonviable seedlings, no.	Nonviable seedlings, %	Hygromycin-sensitive seedlings, %	χ^2 (C1 × wild type)
C1 selfed	−	334	6	1.8		0.08†
C1 × wild type	−	176	3	1.7		
C1 × A^+	−	148	6	4.1		0.88†
C1 × A^o	−	163	10	6.1		3.38†
C1 selfed	+	677	9	1.3	0	0.252†
C1 × wild type	+	479	9	1.9	0.2	
C1 × A^+	+	391	38	9.7	5.6	24.4‡
C1 × A^o	+	283	51	18.0	11.9	61.7‡

*Reciprocal crosses gave similar results, thus numbers were combined.
†Not significant.
‡Significant at $P < 0.001$.

plants, a plant with progeny positive for the transgene in 31 cases out of 31 analyzed was identified. This met the expectation for a homozygous parent in the case of a single transgenic locus. Seeds of this line were further propagated by selfing for two generations, assuming stability of the homozygosity. However, the amplified seed stock in fact accumulated individuals without the *hpt* inserts. Earlier observation of DNA rearrangements within the complex A locus and partial loss of the insert (12) indicated a possible inherent physical instability of the transgenic DNA as a reason for the appearance of "empty" genotypes. An alternative explanation of the observed instability might lie in a karyotype abnormality and consequent unexpected genetic transmission. However, this seemed unlikely since line A^+ was morphologically indistinguishable from the wild type, and characters like fertility, duration of growing period, seed size, number of chloroplasts in the guard cells of stomata, and pollen grain size—shown to be indicative for variation of chromosome number in *Arabidopsis* (17)—were not significantly different. Nevertheless, when the putative homozygous line A^+ was subjected to chromosome staining of root tip meristems, the chromosome number turned out to be 20, whereas the nontransformed wild type had the 10 chromosomes expected for a diploid. The transgenic line was, therefore, most probably tetraploid.

This finding is consistent with the observed segregation of "empty" plants in the course of the seed amplification. Assuming the formation of diploid gametes and relatively tight linkage of the insert to the centromers, a parental plant of the duplex type ($A^+A^+A^oA^o$) would segregate 35:1 for plants containing the insert, and the triplex type ($A^+A^+A^+A^o$) would produce only progeny containing the transgene. Both cases are in accordance with the 31 individual progeny plants containing the transgene in the S3 and with the appearance of more "empty" plants after two further generations (the expected ratio in the S4 is 16.3% for the duplex type and 2.8% for the triplex type).

The chromosome analysis was extended to the other lines involved in the genetic experiments. A^o, which segregated from the same original transformant as A^+, also had 20 chromosomes, whereas C1 was diploid. Thus, it was necessary to reinterpret the crosses described in Table 1, taking into account the karyotype of the parental plants involved. The crosses between C1 and the wild type, where the expected transgene expression in all hybrid seedlings was found, were crosses between two diploid partners resulting in diploid progeny. In contrast, crosses between C1 and either A^+ or A^o, which yielded 6–12% sensitive hybrid seedlings, were crosses between diploid and tetraploid partners, resulting in triploid progeny plants. It was, therefore, conceivable that the increase in chromosome number was responsible for the appearance of sensitive plantlets among the triploid hybrids.

However, A^o was a derivative of a transgenic line. Although its genome contained no sequence homology to the transgene under investigation, other genetic features beside its polyploidy (e.g., somaclonal variation during tissue culture, integration of nonhomologous carrier DNA) distinguishing it from the wild type could not be excluded. To test the assumption that only the increased chromosome number was responsible for the observed suppression effect, we performed further crosses including another tetraploid wild-type line from the same ecotype generated by colchicine treatment.

Several individual plants of C1 as females were crossed with the diploid wild type, the tetraploid strain A^o, and the tetraploid wild type. Results of the selection assay on the hybrid seedlings are summarized in Table 2. The ratio of nonviable seedlings found under nonselective conditions was even lower in this batch of hybrid seeds than in the previously described experiment, and was not significantly different in the three hybrid populations. Variation in size and rate of development of seedlings was more pronounced among the triploid than the diploid hybrid plants, but they remained green and bleaching was not observed in the absence of hygromycin (Fig. 1*c*). Under hygromycin selection, the hybrids between C1 and the diploid wild type showed complete resistance (Fig. 1*a*), whereas the progeny of the diploid C1 with either A^o or the tetraploid wild type included hygromycin-sensitive seedlings with bleaching symptoms (Fig. 1 *b* and *d*). In most cases, these plantlets developed further than nontransformed wild-type seedlings but were arrested at different stages during expansion of cotyledons or growth of the first true leaves. A similar range of phenotypes of hygromycin-sensitive, triploid hybrids was produced when C1 was crossed with A^o or with the tetraploid wild type (Fig. 1 *b* and *d*). Therefore, the loss of hygromycin resistance in the course of somatic growth in a subset of the hybrids was indeed correlated with crosses between a diploid plant containing an active *hpt* gene and a tetraploid partner.

It was important to distinguish whether the loss of hygromycin resistance in triploids resulted from instability of the triploid configuration (early loss of the chromosome carrying the C1 allele) or was indeed due to epigenetic suppression. Several secondary root tips from the triploid hybrids were subjected to chromosome staining, which confirmed that triploidy was stably maintained during several weeks of somatic growth. As previously shown (10), a tissue explant from a plant with a silent transgene may display hygromycin resistance in callus produced in the presence of plant growth factors. Accordingly, triploid progeny seedlings from each type of cross were grown under nonselective conditions for 3 weeks,

Table 2. Suppression of an active transgene by a tetraploid wild-type

Cross	Selection	Seedlings tested, no.	Nonviable seedlings, no.	Nonviable seedlings, %	Hygromycin-sensitive seedlings, %	χ^2 (C1 × diploid wild type)
C1 × diploid wild type ($2n \times 2n$)	−	117	0	0		
C1 × tetraploid A^o ($2n \times 4n$)	−	75	3	4		2.51*
C1 × tetraploid wild type ($2n \times 4n$)	−	58	0	0		
C1 × diploid wild type ($2n \times 2n$)	+	171	0	0	0	
C1 × tetraploid A^o ($2n \times 4n$)	+	120	26	21.6	17.6	38.1†
C1 × tetraploid wild type ($2n \times 4n$)	+	104	12	11.5	11.5	18.0†

*Not significant.
†Significant at $P < 0.001$.

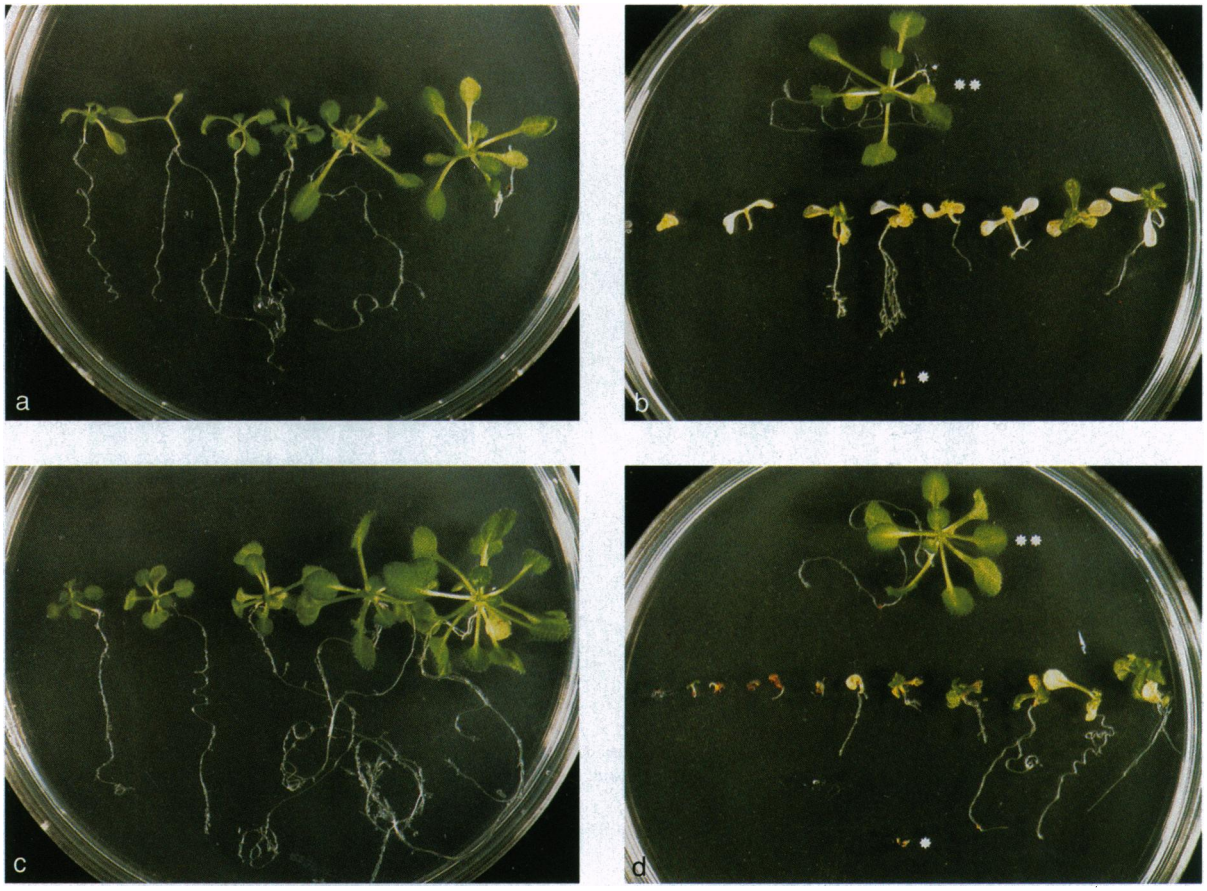

FIG. 1. Phenotypes of hybrid seedlings produced from the crosses C1 × diploid wild type (*a*), C1 × A° (*b*), and C1 × tetraploid wild-type (*c* and *d*) after growth on hygromycin-containing medium (*a*, *b*, and *d*) or nonselective medium (*c*). In *a* and *c*, plantlets were chosen to represent the maximum phenotypic variation. In *b* and *d*, plantlets with signs of sensitivity were selected to delineate the range of phenotypes. * and **, Seedling of the nontransgenic wild type and a fully resistant C1 plantlet, respectively.

and replica cuttings from cotyledons or true leaves of individual plantlets were then grown on callus-inducing medium either with or without hygromycin. All explants from 50 C1 × A^+ triploids, 50 C1 × A° triploids, and 33 C1 × wild type 4*n* triploids showed uniform initiation of callus and similar growth rates on selective medium, indistinguishable from explants of C1 × wild type 2*n* hybrids. Thus, it can be concluded that the transgene of C1 is present in all triploid hybrid plants but reversibly suppressed in a certain subset of the seedling population. The epigenetic character of silencing associated with ploidy changes was recently confirmed by the preliminary analysis of induced tetraploid C1 plants. Polyploidization to 4*n* resulted in even more drastic transgene silencing than that described for triploid hybrids which is transmitted to subsequent generations (O.M.S. and J.P., unpublished results).

Hygromycin sensitivity of seedlings only indicates a low level of transgene expression indirectly, with the threshold between resistance or sensitivity dependent upon the antibiotic concentration. To quantify *hpt* expression more directly, we determined the steady-state level of *hpt* transcripts in Northern blot experiments with RNA from 24 individual, nonselected plants. All plants contained one C1 transgene each, derived from the same parent, but with either one or two chromosome sets coming from a diploid or a tetraploid wild-type parent, and siblings subjected to selection included 0 and 11.5% hygromycin-sensitive seedlings respectively (Table 2). The amounts of *hpt* transcript in the triploid plants were indeed much lower than in diploid plants (Fig. 2). Quantitative evaluation against an internal control (25S rRNA) for each of the 24 plants indicated that the level of *hpt* transcript in the triploid plants in average was reduced to 34.8% of that in diploids. This is much lower than that expected from the relative transgene copy number in hemizygous diploid and triploid genomes. Therefore, the ploidy change and/or other karyotype alterations represent important additional parameters that influence the epigenetic regulation of transgene expression.

DISCUSSION

We have presented evidence for reduced transgene expression in hybrids between diploid and tetraploid plants, leading to a loss of the resistant phenotype at various stages of seedling development in part of the population. This type of suppression was observed for a single copy insert in the absence of other, trans-acting copies of the transgene and is therefore different from homology-dependent gene silencing. The reduction of gene expression was reversible under selective tissue culture conditions, as described before for line A (10). The suppression effect in triploid plants, first observed after crosses with a tetraploid derivative of a transgenic line, was confirmed for progeny of an independently generated nontransgenic tetraploid line. Therefore, an increase in chromosome number can result in epigenetic gene silencing, creating stochastic variations in gene expression patterns.

The formation of polyploids in plants seems to be frequent and successful (18). Even closely related polyploids have multiple evolutionary origins, and studies using molecular markers have indicated that some polyploids have arisen relatively recently, within the last 150 years or even less (19). Nevertheless, although allo- and autotetraploids have a generally higher degree of heterozygosity and allelic diversity,

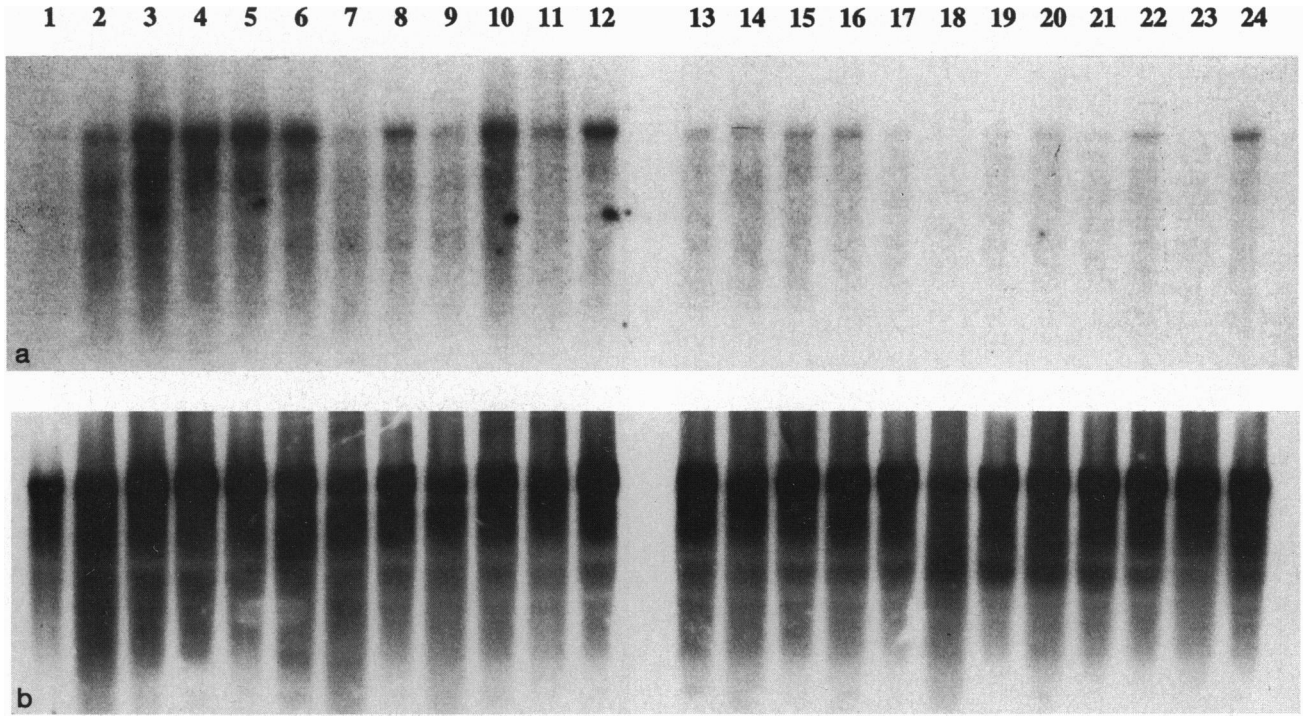

FIG. 2. Northern blot of RNA prepared from progeny hybrid plants of the crosses C1 × diploid wild type (lanes 1–12) and C1 × tetraploid wild type (lanes 13–24), probed with the *hpt* gene (*a*). The same membrane was probed with a gene for 25S RNA from *A. thaliana* (*b*) as an internal standard for the total amount of RNA loaded.

"the number of genes expressed in a polyploid could be sufficiently reduced to return the polyploid to a level of expression similar or identical to that of the diploid ancestor, i.e., the polyploid would become genetically diploidized" (19). This was described as "silencing" of excessive alleles, illustrated by several examples of isozyme patterns—e.g., in a tetraploid fern (20) or in tetraploid *Chenopodium* species (21), and was discussed as a driving force for speciation (22). It is important to notice that such a genetic diploidization is generally assumed to originate from genetic alteration due to gene mutations or meiotic instability, resulting in multiple, nonfunctional alleles. However, it would require a rather high mutation rate to explain the quick genetic diploidization of newly formed polyploids. Such changes have indeed been demonstrated to occur two to five generations after the formation of synthetic polyploids in Brassica (23). The results presented here suggest that some of the nonexpressed "null alleles" could occur even faster—namely, immediately after the change of ploidy—by epigenetic silencing events. Moreover, gene silencing that is initially epigenetic, and therefore reversible, could be a step before entering a genetically fixed, irreversible state. Epigenetic silencing may even accelerate mutagenesis of the affected genes. The extremely rapid occurrence of repeat-induced point mutations in duplicated genes of *Neurospora crassa* (24) might be a prototype for such a process.

It is possible to imagine that silencing is confined to particular genomic regions. A comparison of total protein patterns in haploid, diploid, and tetraploid maize revealed that the levels of many proteins were positively correlated with ploidy, but for other proteins the relationship was inverse (25). A dosage series for various chromosomal arms was seen as evidence for a more frequent negative influence on gene product levels in these segmental aneuploids, with different portions showing stronger or weaker effects, affected by regions assumed to contain regulatory genes (25). However, neither the large rRNA species nor enzymes involved in fatty acid metabolism are dosage compensated in monosomic versus diploid maize plants (26).

Interestingly, one of the most striking epigenetic phenomena shows a clear dosage effect in the comparison of different karyotypes: the frequency of paramutation of a specific paramutagenic allele at the *sulfurea* locus of tomato is different between diploid, triploid, and tetraploid plants and depends on the ratio of paramutagenic to paramutable alleles (27, 28). It was demonstrated further (29) that tetraploid plants initially carrying two paramutable alleles can contain tissue with none, one, or two of these alleles modified by the paramutagenic copies. The allelic interaction, therefore, can occur stepwise and is preceded by some sort of counting of the alleles involved.

The most impressive "counting" and corresponding inactivation is known from eutherian mammals and marsupials, where very many of the genes located on X chromosomes except one are inactivated (1, 30). Which X chromosomes are inactivated is determined in early embryonal development, and subsequently in different tissues (31), and inactivation is maintained throughout the whole life of somatic cells. Some X-linked genes nevertheless escape from inactivation, and position rather than just sequence determines expression (32). Genetic and molecular data indicate that the copy number of a genetic locus called XIC (X inactivation center) is involved in the counting mechanism (33). Although the mechanism of dosage compensation for sex chromosome-linked genes in *Drosophila* is different (34, 35), the trans-acting loci controlling this process are genetically better characterized. Interestingly, multiplication of these loci by polyploidy alters the expression of responsive genes (36, 37). One such locus (*Lip*) that suppresses certain alleles of *white* affects position effect variegation (an epigenetic modification of genes in proximity to heterochromatin) (38). How specificity of suppression in such a system is guaranteed is not yet fully understood.

In addition to ploidy differences among individuals or species, many plants are polysomatic individuals consisting of cells with different amounts of nuclear DNA. Measurements by flow cytometry have indicated that nuclei in some tissues or

organs can contain up to 128 times the haploid genome complement (39). *A. thaliana* is known to have a high level of polysomaty (40–42). Furthermore, the frequency and distribution of cells with different nuclear DNA contents is developmentally regulated (43, 44) and respond to environmental conditions (45). Parallels can also be drawn to the regulation of epigenetic silencing. Various growth conditions (greenhouse versus *in vitro* culture) have been described to influence the frequency of epigenetic silencing in *Nicotiana sylvestris* plants transgenic for a chitinase gene (46). Silencing in this system was also developmentally regulated, as well as reset during meiosis. In view of our results, it is possible that differential gene expression in cells of different ploidy might be involved. Thus, this could represent another example of epigenetic gene regulation coupled to polysomaty.

Transgenic plants are rarely analyzed for their karyotype. Thus instability of transgene expression may be more frequently correlated with variation of chromosome numbers than noticed so far. For example, loss of antibiotic resistance was also described for several transgenic lines of *Nicotiana plumbaginifolia* and *Nicotiana tabacum* (47), but there was only indirect evidence (e.g., differences between outcrossing and selfing, reduced fertility, aberrant seedlings) of karyotype variation. Unexpected segregation of antibiotic markers and subsequent karyotype analysis led to the discovery of aneuploidy (tri- and tetrasomy) in a transgenic tobacco line. In this case, distorted segregation ratios were related to aneuploidy, but originated from the loss and/or gain of transgene copies and not from altered gene expression (48).

If epigenetic gene silencing in plants represents a general response to an increase in chromosome number or the quantity of nuclear DNA, ploidy changes could influence gene regulation substantially. Such regulation could affect specific genes, genomic regions or whole chromosomes, modifying gene expression patterns between cells of an individual organism, between individuals, and between populations. This may contribute significantly to the adaptation potential of plants and could have a strong impact on plant evolution and plant breeding.

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