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Non-Mendelian Inheritance of Macronuclear Mutations Is Gene Specific in *Paramecium tetraurelia*†

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Paramecium tetraurelia **contains two types of nuclei, a diploid germinal micronucleus and a large transcriptionally active macronucleus. The macronuclear genome is formed from the micronuclear DNA during sexual reproduction. Previous studies have shown that the processing of the A-type variable surface protein gene during formation of a new macronucleus is dependent on the presence of the A gene in the old macronucleus. It is not clear if this is a general feature that controls the formation of the *Paramecium* macronuclear genome or a unique feature of the A locus. Using micronuclear transplantation, we have constructed a strain that has a wild-type micronucleus but has macronuclear deletions of the A- and B-type surface protein genes. Neither the A nor the B gene is incorporated into the new macronucleus after sexual reproduction. Macronuclear transformation of this strain with the B gene rescues the B-gene deletion after formation of the next macronucleus but has no effect on the A deletion. Similarly, transformation with the A gene shows gene-specific rescue for A but not B. The effect of the old macronucleus on the processing of the new macronucleus results in a pattern of non-Mendelian inheritance of both macronuclear deletions. Progeny from the wild-type exconjugant are all wild type, and progeny from the A^- B^- exconjugant are mutant. The features of this A^- B^- non-Mendelian mutant demonstrate that the regulation of macronuclear DNA processing is gene specific, and our results open the possibility that this type of regulation affects many regions of the *Paramecium* genome.**

The ciliated protozoan *Paramecium tetraurelia* possesses two kinds of nuclei within a single cell. The highly polyploid macronucleus is transcriptionally active and therefore determines the phenotype of the cell. In contrast, the two diploid micronuclei are transcriptionally silent but participate in the nuclear reorganization events of autogamy (self-fertilization) and conjugation (reciprocal exchange of nuclei with cell of the opposite mating type). During either of these events, a new macronuclear genome is produced from the micronuclear genome through a process which involves extensive amplification and rearrangement of micronuclear DNA. This process of macronuclear development results in differences in the genetic content of the two types of nuclei.

The previously isolated mutant d48 provides an example of how the macronuclear content in the *Paramecium* cell can differ from the micronuclear content. Originally isolated after X-ray mutagenesis (2), the d48 mutant is unable to express surface protein A, one of a family of surface proteins in *P. tetraurelia* (reviewed in references 1 and 14). Previous experiments have shown that although d48 contains the A gene in its micronuclei, the gene is either absent in the macronucleus or present in extremely low copy numbers (2, 8).

A striking feature of the d48 mutant is its unusual genetic behavior. A cross between two paramecia yields F_1 heterozygous cells with identical micronuclear genotypes. Therefore, a cross between a typical Mendelian mutant and a wild-type cell produces identical heterozygous progeny cells from both the wild-type parent and the mutant parent. The F_1 progeny which result from a cross between d48 and wild-type cells have genetically identical micronuclear genotypes, but all the progeny derived from the wild-type parent are phenotypically A^+ and progeny from the mutant are A^-. This pattern of "cytoplasmic" inheritance continues into the F_2 generation. A normal Mendelian mutation would segregate 1:1 in the F_2 generation regardless of the parental cytoplasm, but the A^- mutation is present only in F_2 cells derived from the d48 parent, while all F_2 cells derived from the wild-type parent are wild type. Since both wild-type and d48 cells contain a wild-type A gene in their micronuclei, the difference between the progenies must result from a difference in the macronuclear DNA processing events that occur in each cell. When the term non-Mendelian is used below, it refers to this effect of the old macronucleus on DNA processing and the resulting phenotype.

The most intriguing property of the d48 mutant is its ability to be permanently rescued after formation of a new macronucleus if the cloned A gene is transformed into the old macronucleus (5, 10). Recent experiments have shown that the entire A gene is not necessary for d48 rescue; in fact, relatively small portions of the A gene can produce permanent rescue after nuclear reorganization (6, 24). Although it has not been possible to localize the rescue activity to one specific region of the A gene, rescue appears to be gene specific, since even the closely related B surface protein gene cannot rescue d48 (24).

In 1990, Kobayashi and Koizumi (8) created a line designated d48* by replacing the micronuclei in d12, a Mendelian A^- mutant which contains both micronuclear and macronuclear A-gene deletions, with wild-type micronuclei. When crossed with wild-type cells, the d48* line produced the same inheritance pattern as the d48 mutant: all progeny derived from the wild-type parent were wild type, and all progeny derived from the d48 parent were A^-. If macronuclear DNA

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processing generally requires a copy of each gene in the old macronucleus, then it should be possible to create cell lines which exhibit d48-like properties for other genes. Specifically, we predicted that a mutant carrying a complete deletion of a gene in its macronucleus would not be able to process the gene into the next macronuclear generation, even if the micronucleus of the mutant were completely normal. Obviously, to test this hypothesis, we had to choose a gene that is not essential for viability. The B surface protein gene is unlinked to the A locus, is not required for cell viability, and was recently cloned and sequenced. A recent genetic analysis of all available B^- macronuclear deletion mutants did not reveal any that were inherited in the same manner as the A gene in d48 (17); thus, the B locus was a good candidate gene with which to test our theory.

In this study, we replaced the micronuclei of a Mendelian $A^- B^-$ deletion mutant with wild-type micronuclei, creating a cell line which has a deletion for both the A and B genes in the macronucleus but has a wild-type micronucleus. Genetic and molecular analyses of the renucleated cell line showed that it has the same characteristics as d48, both in its non-Mendelian inheritance pattern and in its ability to be permanently rescued after macronuclear transformation with both the A and B genes. This new mutant provides the first evidence that the macronuclear processing defect for the A gene seen in d48 may be a defect in a basic mechanism that controls the formation of much of the *Paramecium* macronuclear genome.

MATERIALS AND METHODS

Cell lines, cultivation, and tests for phenotype. *P. tetraurelia* stock 51 is homozygous for both the A and B genes. d12.141 was originally derived from the Mendelian A^- mutant d12. It contains both micronuclear and macronuclear deletions of the A surface protein gene (13, 15) as well as a complete macronuclear deletion of the B surface protein gene (17). It contains the genetic marker *twisty* (*tw*), a morphological mutation, and a Mendelian *trichocyst nondischarge* (*ND*) (19) mutation. Because cells renucleated with micronuclei from wild-type cells acquired the trichocyst discharge trait after formation of a new macronucleus (see Table 1), wild-type stock 51 cells used in genetic crosses were marked with a Mendelian *ND* mutation. Trichocyst discharge was tested by mixing 10 μl of cells in culture fluid with 10 μl of saturated picric acid and examining the cells at a magnification of ×400. This treatment induces wild-type cells to extrude their trichocysts, resulting in a halo of trichocysts around the cell body. All cells were cultured in 0.25% wheat grass medium buffered with sodium phosphate. The medium was inoculated with a nonpathogenic strain of *Klebsiella pneumoniae* 1 or 2 days prior to being used.

Micronuclear transplantation. Nuclei were removed with a micromanipulator with a single needle by the methods of Koizumi (9) and Mikami and Koizumi (12). Since *P. tetraurelia* contains two micronuclei, two steps of manipulation were necessary. The first micronucleus was removed from the cell four to five fissions after autogamy (micronuclei are most easily visualized under the phase-contrast microscope during logarithmic growth), and the remaining micronucleus was removed four to five fissions later. After enucleation, a few cells from each clone were examined by aceto-orcein stain to ensure that the clone was amicronucleate. Cells were then cultivated for 2 days, and a new micronucleus was implanted. Several days after micronuclear implantation, cells were starved to induce autogamy (self-fertilization), which restored the cell to the normal two-micronucleus complement. (The first step of autogamy involves meiotic division to produce eight haploid products [four in a cell with one micronucleus], but only one of these products survives to go on and produce the zygotic nucleus.) Following autogamy and additional cultivation, both the absence of the twisty phenotype and the presence of the trichocyst discharge trait were used to verify that the clone had received a wild-type micronucleus.

Genetic crosses. Mating and induction of autogamy were carried out as described by Sonneborn (18). All laboratory stocks used were homozygous clones resulting from recurring autogamy. A cross between two paramecia yields heterozygous F_1 exconjugant clones with identical micronuclear genotypes. F_2 lines are obtained by inducing autogamy in the F_1 clones. For each gene locus, half of the resulting F_2 lines are homozygous for the allele found in one parent and the other half are homozygous for the allele found in the other parent. Hence, a normal Mendelian mutation would segregate with a 1:1 ratio in the F_2 generation. Both the Mendelian marker *ND* (19) and the A serotype were used to distinguish the two parents and to indicate the proper exchange of nuclei. Expression of the A serotype can be used in this manner because of the stability of its expression. Wild-type parents expressed the A serotype at the time of mating, and F_1 clones from the wild-type side of the cross continued to express the A surface protein. True conjugation was confirmed by the appearance of the trichocyst discharge trait in F_1 cells from both sides of the cross.

Microinjection. Microinjections of DNA were performed as previously described by Godiska et al. (4). Prior to microinjection, renucleated cell lines (M cells) were brought through autogamy by the daily isolation method (18) and allowed to grow for at least two fissions. Newly formed macronuclei were injected with either pSB11.6BX, a pUC119 plasmid containing the entire coding region of the surface protein B gene as well as 3 kb of upstream and 2 kb of downstream noncoding sequence, or pSA10XS, a pUC119 plasmid containing the entire coding region of the A gene as well as 1.5 kb of upstream and approximately 300 bp of downstream noncoding sequence (23). Plasmid DNA was dissolved in buffer previously described by Tondravi and Yao (22) to a final concentration of 1 to 2 mg/ml. Between 3 and 6 pl of this solution was injected into each cell with a glass microneedle 1 to 2 μm in diameter at the tip. Following microinjection, each cell was placed in 0.75 ml of fresh medium and cultured at 27°C.

Preparation of DNA from *P. tetraurelia*. Large-scale DNA isolations were performed as follows. Packed cells (0.1 to 0.2 ml) from 8-liter cultures were resuspended in 0.7 ml of culture fluid and then quickly squirted into 2.1 ml of lysing solution (10 mM Tris-HCl [pH 9.5], 50 mM sodium EDTA, 1% sodium dodecyl sulfate [SDS]) at 65°C. After 10 min, 7 ml of saturated CsCl was added and the solution was centrifuged in a vTi65.1 rotor at 55,000 rpm for approximately 20 h. Fractions containing DNA were collected and dialyzed overnight against Tris-EDTA. Small-scale DNA isolations were performed on F_1 and F_2 cell lines as follows. One-hundred-milliliter cultures of each cell line were pelleted, resuspended in 0.4 ml of culture fluid, and quickly squirted into 0.8 ml of lysing solution at 65°C. After 10 min, lysates were extracted with phenol-chloroform and precipitated in 2 volumes of ethanol. Collected pellets were resuspended in Tris-EDTA, treated with RNase A followed by proteinase K, and then extracted twice more with phenol-chloroform before a final ethanol precipitation.

Hybridizations. Southern blot analyses were performed according to the method of Maniatis et al. (11). Because of the vast difference in the gene copy numbers of the macronucleus (more than 1,000) and the micronuclei (a total of four from two diploid micronuclei), total genomic DNA examined by Southern blot hybridizations with *P. tetraurelia* is essentially

TABLE 1. Phenotypes of strains for micronuclear transplantation

Strain	Mendelian markers	Serotype
Stock 51, wild type (donor of micronucleus)	*D*, *tw*$^+$	A$^+$ B$^+$
d12.141 (amicronucleate recipient)	*ND*, *tw*	A$^-$ B$^-$
M line (recipient after autogamy)	*D*, *tw*$^+$	A$^-$ B$^-$

macronuclear DNA. Filters were washed in a solution containing 10× Denhardt's solution, 0.1% SDS, 0.2 M phosphate buffer, and 5× SET (1× SET is 0.15 M NaCl, 30 mM Tris, and 2 mM EDTA) at 65°C for 1 h. The filters were then incubated in hybridization solution (1× Denhardt's solution, 20 mM phosphate buffer, 5× SET, 0.25% SDS) for 1 h at 65°C before a labelled probe was added. After an overnight incubation, filters were washed three times for 30 min each in a solution containing 0.2× SET, 0.1% SDS, 0.1% sodium PP_i, and 25 mM phosphate buffer at high-stringency temperature (71 to 72°C).

Scoring for rescue of M-cell lines. Each injected cell was grown for 3 days at 27°C, at which time wells had usually reached a density of 500 to 1,000 cells. Fifty microliters of cells was removed from each well (approximately 50 cells) and scored for either surface protein A expression (when injected with pSA10XS) or surface protein B expression (when injected with pSB11.6BX). Cells were considered transformed if expression was found, even if less than 100% of the cell line showed expression. Uninjected control cells were also routinely checked for A and B expression, but no expression of either surface protein was ever found. Selected transformed cell lines were then taken through autogamy to induce the formation of new macronuclei. Following autogamy, cells were grown to 100-ml cultures for DNA isolation. Genomic DNA was digested with *Hin*dIII, blotted to nitrocellulose, and simultaneously probed with nick-translated pSA1.4H and pSB2.1H. Cells were considered rescued for A if they contained the 1.4-kb *Hin*dIII fragment of the A gene and rescued for B if they contained the 2.1-kb *Hin*dIII fragment of the B gene on a Southern blot.

RESULTS

Micronuclear transplantation. To determine if the old macronuclear copy of the B surface protein gene is required for its correct processing into the next macronucleus, we first replaced the micronuclei of the cell line d12.141 with wild-type micronuclei (see Materials and Methods). Originally derived from d12, the d12.141 mutant contains complete macronuclear deletions of both the A and B genes according to Southern blot analysis. We have previously determined that the d12.141 mutant inherits both the A and B genes in a Mendelian fashion. A cross with wild-type cells yields a Mendelian segregation pattern for the A and B genes in the F_2 generation with no detectable influence of parental cytoplasm (1:1:1:1 ratio of A$^+$ B$^+$:A$^-$ B$^+$:A$^+$ B$^-$:A$^-$ B$^-$) (17). The d12.141 mutant carries both the *tw* marker and the *ND* mutation as genetic markers. Following micronuclear transplantation and autogamy, three separate renucleated cell lines, M1, M2, and M3, were able to discharge their trichocysts when treated with picric acid and the twisty phenotype was no longer observed. Since the transplanted micronuclei were both *trichocyst discharge* (*D*) positive and *tw*$^+$, this indicated that the cells contained normal wild-type micronuclei (Table 1).

Renucleated cells cannot process the A or B gene into their developing macronuclei. To determine whether renucleated cells contained the A and B genes in their macronuclei after autogamy, a 100-ml culture of each clonal line was grown for genomic DNA isolation. Total genomic DNAs were digested with *Hin*dIII, electrophoresed on an agarose gel, and blotted to nitrocellulose. Blots were simultaneously probed with nick-translated pSA1.4H, a plasmid containing a 1.4-kb *Hin*dIII fragment of the A gene, and pSB2.1H, a plasmid containing a 2.1-kb *Hin*dIII fragment of the B gene. Probed filters were washed under stringent conditions (see Materials and Methods) so that the closely related A and B genes would not cross-hybridize. Figure 1 shows that neither the A nor the B gene is detectable in total genomic DNA from the renucleated cell line M1 after autogamy (lanes 1 and 2). Identical results were obtained with the M2- and M3-cell lines. Because of the large copy number of genes in the macronucleus (more than 1,000) compared with that of the diploid micronuclei, micronuclear genes are not detectable in Southern blots of total genomic DNA.

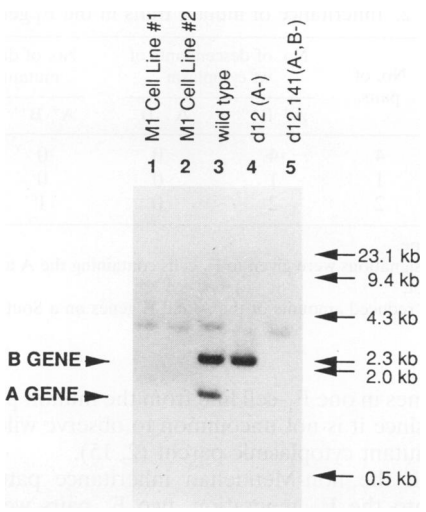

FIG. 1. Genomic Southern analysis of the renucleated cell line M1. Total genomic DNA was obtained from two single-cell isolate lines, both derived from postautogamous M1 cells (lanes 1 and 2). DNA was digested with *Hin*dIII, electrophoresed, blotted, and then simultaneously probed with pSA1.4H and pSB2.1H, two plasmids that contain *Hin*dIII fragments from the A and B genes, respectively. The A and B genes are not detectable in M1 total genomic DNA, which consists almost entirely of macronuclear DNA. The less intense 3.8-kb band results from cross-hybridization with another sequence related to the B gene (16). Lane 3, wild-type stock 51 DNA; lane 4, d12 (A$^-$) DNA; lane 5, d12.141 (A$^-$ B$^-$) DNA.

The inheritance of the A and B genes is non-Mendelian in M-cell lines. In the d48 mutant, inheritance of the A gene is influenced by the cytoplasm. If the B gene is under similar control in the M-cell lines, then it should also be influenced by the cytoplasm. We crossed each renucleated cell line with wild-type cells and looked for the presence of the A and B genes in F_1-cell lines by using Southern blots. Normally, the inheritance pattern of the d48 mutant is obvious in the F_1 generation, since F_1 cells derived from the cytoplasm of the wild-type parent are wild type, while F_1 cells derived from the cytoplasm of the d48 parent are A$^-$. Of seven F_1 pairs examined, all but one pair showed a cytoplasmic inheritance pattern for both the A and B genes. One pair appeared to contain reduced amounts of the A and B genes in F_1 cells derived from mutant cytoplasm (Table 2). The presence of the

TABLE 2. Inheritance of mutant traits in the F_1 generation

Cross[a]	No. of pairs	No. of descendants of wt cytoplasm		No. of descendants of mutant cytoplasm	
		$A^+ B^+$[b]	$A^- B^-$	$A^+ B^+$[b]	$A^- B^-$
M1 × wt	4	4	0	0	4
M2 × wt	1	1	0	0	1
M3 × wt	2	2	0	1[c]	1

[a] wt, wild type.
[b] $A^+ B^+$ designations were given to F_1 cells containing the A and B genes on a Southern blot.
[c] Contained reduced amounts of the A and B genes on a Southern blot.

A and B genes in one F_1-cell line from the mutant parent is not surprising since it is not uncommon to observe wild-type cells from the mutant cytoplasmic parent (2, 15).

To see if the non-Mendelian inheritance pattern would continue into the F_2 generation, two F_1 pairs were brought through autogamy to produce an F_2 generation. Because induction of the B serotype is time-consuming and not always reliable, all F_2 progeny were analyzed by the same Southern blot procedure described above. For both pairs, all of the progeny derived from wild-type cytoplasm contained both the A and B genes, while all of the progeny derived from mutant cytoplasm were missing the A and B genes. If one performs a chi-square test based on the hypothesis of a normal Mendelian inheritance pattern (1:1:1:1 ratio of $A^+ B^+$:$A^- B^+$:$A^+ B^-$:$A^- B^-$) for the A and B genes in F_2 progeny, the chi-square values, which range from 42.7 to 45, are completely unacceptable for Mendelian inheritance, with *P* values of less than 0.005. On the other hand, using the chi-square test on *ND* marker segregation (1:1 ratio of *D* to *ND*) results in perfectly acceptable chi-square values which range from 0 to 1.32, with *P* values greater than 0.1 (Table 3).

Control of macronuclear formation is gene specific. To determine if the cloned B gene can permanently rescue M cells in the same manner as the A gene rescues d48, we microinjected M1 cells with pSB11.6BX, a plasmid which contains the entire coding region of the B gene as well as 3 kb of upstream sequence and 2 kb of downstream sequence. Cells were allowed to grow by vegetative fission for 3 days after microinjection (to approximately 1,000 cells). Transformants were then selected on the basis of B expression by using anti-B serum. Transformed cell lines were starved to induce autogamy, and postautogamous cultures were grown for DNA isolation. DNA was digested with *Hin*dIII, blotted to nitrocellulose, and simultaneously probed with a nick-translated 2.1-kb *Hin*dIII fragment from the B gene and a 1.4-kb *Hin*dIII fragment from the A gene. Following overnight hybridization, filters were stringently washed so that the two probes would not cross-hybridize. Figure 2 shows the results with two transformed lines both before (lanes 3 and 4) and after (lanes 5 and 6) autogamy. In both these examples, transformation by microinjection of the cloned B gene resulted in permanent rescue of the B mutation. Results with six additional transformed cell lines were identical (Table 4). In most cases, cells continued to express surface protein B even after autogamy. Despite the ability of the cloned B gene to rescue the B-gene deletion in the M-cell line, it had no effect on the deletion of the A gene (Fig. 2 and Table 4).

To determine if the A gene would also show gene-specific rescue in M-cell lines, we microinjected pSA10XS, a plasmid which contains the entire coding region of the A gene as well as 1.5 kb of upstream and 300 bp of downstream noncoding sequence. Following microinjection and 3 days of growth, transformants were selected on the basis of protein A expression by using anti-A serum. Following autogamy, DNA from nine transformed cell lines was isolated, digested with *Hin*dIII, and blotted to nitrocellulose. The filters were probed with both an A-gene probe, pSA1.4H, and a B-gene probe, pSB2.1H (Fig. 2). Seven of the nine cell lines were rescued for the A gene following autogamy, as indicated by the presence of the 1.4-kb *Hin*dIII fragment of the A gene (Table 4). This 78% rescue efficiency is not unusual for the pSA10XS A-gene plasmid. Similar rescue efficiencies have been obtained for microinjection of pSA10XS into the d48 cell line (24). None of the nine cell lines transformed with pSA10XS, however, contained the 2.1-kb *Hin*dIII fragment of the B gene following autogamy. Control of the B-gene mutation is therefore also gene specific since microinjection of the cloned A gene had no effect on the B-gene mutation.

DISCUSSION

In this paper, we demonstrate that the macronuclear DNA processing of the B locus is defective in a strain that contains a normal micronucleus and a deletion of the B gene in the old macronucleus. This $A^- B^-$ strain has the same features as the previously studied d48 mutant: the phenotype is inherited according to the cytoplasm of the parental cell, and the deletion of the macronuclear gene can be rescued during subsequent sexual events if the old macronucleus is transformed with the corresponding cloned gene. Until now, no mutations affecting the B locus whose inheritance is influenced by the cytoplasm have been reported.

The extent to which the old macronucleus controls the formation of the genome in the new macronucleus is not clear. It is possible to argue that since the B gene is closely related to the A gene (73% nucleotide sequence identity), we should not

TABLE 3. Inheritance of mutant traits in the F_2 generation

Cross and descendant cytoplasm	No. of descendants with serotype				χ^{2a}	*P*	Marker segregation		χ^{2a}	*P*
	$A^+ B^+$	$A^- B^+$	$A^+ B^-$	$A^- B^-$			*ND*	*D*		
M1 × wt										
wt	20	0	0	0	45	<0.005	11	9	0.2	>0.9
mutant	0	0	0	19	42.7	<0.005	12	7	1.32	>0.1
M3 × wt										
wt	19	0	0	0	42.7	<0.005	12	7	1.32	>0.1
mutant	0	0	0	20	45	<0.005	10	10	0	>0.9

[a] χ^2 values were calculated with an expected ratio of 1:1:1:1 for the A and B genes and of 1:1 for marker segregation.

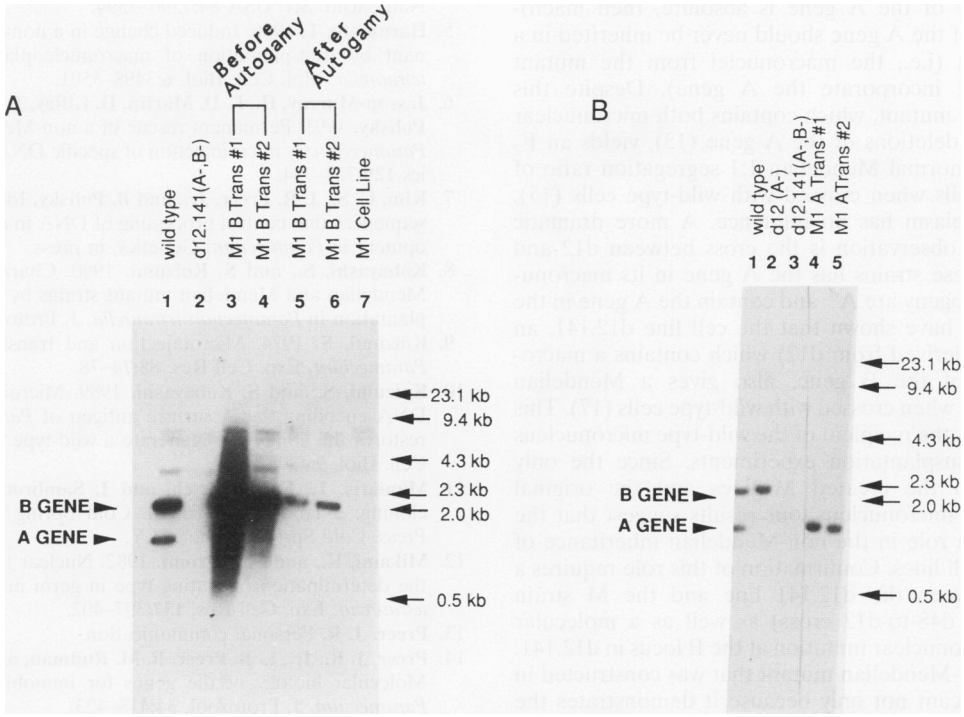

FIG. 2. Genomic Southern analysis of transformants demonstrates gene-specific rescue of the M1-cell line. (A) M1 cells were transformed with pSB11.6BX, a plasmid which contains the entire coding region of the B gene as well as 5′ and 3′ flanking sequences. DNA was obtained from each transformant both before (lanes 3 and 4) and after (lanes 5 and 6) autogamy. The DNA was digested with *Hin*dIII, electrophoresed, blotted, and simultaneously probed with a 1.4-kb *Hin*dIII fragment from the A gene and a 2.1-kb *Hin*dIII fragment from the B gene. The strong signals in the lanes of the before-autogamy lines are typical of cell lines transformed with a high copy number of injected DNA. The presence of the B gene after autogamy indicates that the cell was able to process the gene into the macronucleus from the wild-type micronucleus, since injected DNA is lost after nuclear reorganization. Lane 1, wild-type stock 51 DNA; lane 2, d12.141 (A^- B^-) DNA; lane 7, uninjected M1 cells. (B) M1 cells were transformed with pSA10XS, a plasmid that contains the entire coding region of the A gene as well as 5′ and 3′ flanking sequences (23). DNA was isolated from transformed cells after autogamy and then treated and probed identically as described for panel A. Lane 1, the wild type; lane 2, d12 (A^-); lane 3, d12.141 (A^- B^-); lanes 4 and 5, transformed cell lines after autogamy. Before autogamy, transformed cell lines were identified by expression of the A surface protein.

be surprised that its inheritance is influenced by the old macronucleus. Nevertheless, this argument ignores the most remarkable feature of the A^- B^- non-Mendelian cell line: the DNA processing defect is gene specific, yet rescue of this defect occurs in *trans* through some type of cytoplasmic signal. Despite the similarity of the A and B genes, they are controlled independently during macronuclear development. This was demonstrated by the ability of the cloned A gene to rescue the A but not the B deletion after formation of a new macronucleus in the M-cell line. Both this report and other experiments with the d48 mutant have shown that transformation with the B gene is not capable of rescuing the A gene after autogamy (24). Gene specificity has also been demonstrated in the d48 mutant by using the G gene from *Paramecium primaurelia*. Although the *156G* gene is 79% identical to A at the nucleotide level, it cannot rescue the A-gene defect in d48 (7). These data suggest that the presence of each gene in the old macronucleus acts in a nucleotide sequence-specific manner to signal the developing macronucleus. If a gene is not present in the old macronucleus, it cannot be incorporated into the developing macronucleus. Other examples of mutants containing macronuclear deletions are necessary to explore the generality of this phenomenon. It is possible that gene location has a role in this regulatory mechanism. Both the A and B genes are located near the end of a macronuclear chromosome (telomere) (3, 17). It is not clear whether genes in internal regions are regulated by the same system.

TABLE 4. Rescue by macronuclear transformation

DNA injected	No. of transformed lines (before autogamy)	No. of rescued lines (after autogamy)	
		A gene	B gene
pSB11.6BX	8	0	8
pSA10XS	9	7	0

The A and B genes are not the only examples of non-Mendelian inheritance in paramecia. The inheritance of mating type and a mutation affecting the discharge of trichocysts (20, 21) also follow a non-Mendelian pattern of inheritance. Sonneborn showed that once a macronucleus is determined for one of these traits, the trait is stable and cannot be altered unless a new macronucleus is made. In addition, the old macronucleus determines the formation of the new macronucleus through a cytoplasmic determinant. Unfortunately, since the genes for these traits have not been cloned, it is not yet known if they will exhibit the same molecular properties or will be capable of the self-rescue that is seen with d48.

It is clear from previous studies that the A gene in the old macronucleus is required for the correct macronuclear processing of the A gene in d48 cells. If this requirement for a

macronuclear copy of the A gene is absolute, then macronuclear deletions of the A gene should never be inherited in a Mendelian fashion (i.e., the macronuclei from the mutant parent should not incorporate the A gene). Despite this prediction, the d12 mutant, which contains both micronuclear and macronuclear deletions of the A gene (13), yields an F_2 generation with a normal Mendelian 1:1 segregation ratio of A^+ cells to A^- cells when crossed with wild-type cells (15). The parental cytoplasm has no influence. A more dramatic illustration of this observation is the cross between d12 and d48. Neither of these strains has the A gene in its macronucleus, yet the F_1 progeny are A^+ and contain the A gene in the macronucleus. We have shown that the cell line d12.141, an $A^- B^-$ cell line (derived from d12) which contains a macronuclear deletion of the B gene, also gives a Mendelian inheritance pattern when crossed with wild-type cells (17). This cell line was used as the recipient of the wild-type micronucleus in our nuclear transplantation experiments. Since the only difference between the created M lines and the original d12.141 line is the micronucleus, our results suggest that the micronucleus has a role in the non-Mendelian inheritance of the B gene in M-cell lines. Confirmation of this role requires a genetic cross between the d12.141 line and the M strain (analogous to the d48-to-d12 cross) as well as a molecular analysis of the micronuclear mutation at the B locus in d12.141.

The $A^- B^-$ non-Mendelian mutant that was constructed in this study is significant not only because it demonstrates the gene-specific nature of non-Mendelian inheritance but also because it will be useful as a tool to study macronuclear DNA processing. A molecular and genetic comparison of non-Mendelian mutations affecting the A and B genetic loci should lead to a better understanding of the rules that govern macronuclear development, as well as opportunities to test models based on information from previous studies.

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