Copyright © 1994 by the Genetics Society of America

The Role of Macronuclear DNA Sequences in the Permanent Rescue of a Non-Mendelian Mutation in *Paramecium tetraurelia*

Yun You, Jill Scott and James Forney

Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907-1153

Manuscript received August 3, 1993
Accepted for publication December 15, 1993

ABSTRACT

The *Paramecium tetraurelia* mutant called d48 has a complete copy of the *A* surface protein gene in its micronuclei, but lacks the *A* gene in the macronucleus. Previous experiments have shown that microinjection of a plasmid containing the entire *A* gene or a large portion of the gene into the macronucleus of d48 rescued the cell line after formation of a new macronucleus (autogamy). Here we show that several different regions of the *A* gene can rescue d48, but 100% of the activity cannot be localized to a single, defined region. Inversion of a sequence contained within an *A* gene plasmid had no measurable effect on rescue efficiency and co-injection of two different plasmids results in enhancement of rescue activity despite the non-contiguous form of the DNA sequences. Both these results suggest that no specific product (RNA or protein) with defined end points is made from the rescuing fragment. A unique restriction site was created in the *A* gene and used to demonstrate that the injected DNA does not serve as a direct template for the synthesis of the new macronuclear DNA. Models to explain the action of the injected DNA are discussed.

THE free-living, single-celled eucaryote Paramecium has two types of nuclei: a highly polyploid macronucleus, which is transcriptionally active and therefore determines the phenotype of the cell, and two micronuclei, which are transcriptionally silent but contain the germline DNA. During sexual reproduction, either autogamy (self-fertilization) or conjugation (mating), the old macronucleus is destroyed and a new one is created from a copy of the micronuclear genome. It is during this process of macronuclear development that extensive cleavage, rearrangement, telomere addition and amplification of the macronuclear DNA occurs, resulting in two functionally distinct types of nuclei which differ in their DNA content (reviewed in BLACKBURN and KARRER 1986, YAO 1989). Differences between micronuclear and macronuclear DNA have been characterized in many ciliates, but little is understood about the molecules that interact with DNA to control these differences. An unusual Paramecium mutant, called d48, has been a useful model to investigate at least one component important in the formation of the Paramecium macronuclear genome. Originally generated by X-ray mutagenesis of *Paramecium tetraurelia* stock 51, the d48 mutant contains a normal copy of the *A* surface protein gene in its micronucleus, but the gene is either deleted or is present in extremely low copy numbers in its macronucleus so that the mutant is unable to express the A surface protein (EPSTEIN and FORNEY 1984, RUDMAN *et al.* 1991). The *A* gene is normally located 8–26 kilobases from the macronuclear telomere and in d48 a new macronuclear telomere is created at the 5′ end of the gene (FORNEY and BLACKBURN 1988).

Genetic experiments have shown that differences between the cytoplasm of d48 and wild-type cells affect the processing of the *A* gene during macronuclear development. Unlike Mendelian mutations which segregate 1:1 in the F_2 generation, a cross between wild-type stock 51 (A^+) and d48 (A^-) cells produces all A^+ cells from wild-type cytoplasm and all A^- cells from d48 cytoplasm (EPSTEIN and FORNEY 1984; RUDMAN *et al.* 1991). The transfer of macronucleoplasm from a wild-type cell into the d48 mutant demonstrated that the old macronucleus is the source of the cytoplasmic determinant (HARUMOTO 1986). Micronuclear transfer experiments have shown that the micronucleus of the d48 cell is completely normal. Transfer of a d48 micronucleus into an amicronucleate wild type cell results in a wild type cell line after autogamy, and the reciprocal transfer of a wild type micronucleus into an amicronucleate d48 cell has no effect on the d48 mutant phenotype after autogamy (KOBAYASHI and KOIZUMI 1990). Together these experiments indicate that cytoplasmic factors produced from the wild type macronucleus during sexual reproduction are necessary for proper incorporation of the *A* gene into the next macronucleus.

Recent studies have shown that microinjection of a plasmid containing the entire *A* gene or even a portion of the gene into the d48 macronucleus results in permanent rescue of the mutation after the formation of a new macronucleus (YOU *et al.* 1991; JESSOP-MURRAY *et al.* 1991). To elucidate the molecular mechanism responsible for the d48 phenomenon, we have constructed several plasmids containing different portions of the *A* gene. These plasmids were microinjected into the d48

macronucleus and their ability to rescue d48 after the formation of a new macronucleus was measured.

MATERIALS AND METHODS

Cell lines and culture: *P. tetraurelia*, stock 51 (ATCC 30303), and strain d48 of *P. tetraurelia* (derived from stock 51) were cultured in 0.25% wheat grass medium (Pines International, Lawrence, Kansas) buffered with 0.45 g of Na_2HPO_4 per liter. In some cases, the medium was augmented with 5 mg of stigmasterol (Sigma) per liter. The sterilized medium was inoculated 24 to 48 hr before use with a nonpathogenic strain of *Klebsiella pneumoniae* (ATCC 27889). Induction of A surface protein expression, assessment of autogamy, harvesting of cells, and detection of the A surface protein by anti-sera testing have been described by SONNEBORN (1950).

Cloned DNA: The nucleotide sequence positions shown in this paper begin numbering with the translation start of the *A* gene as +1 (GenBank accession number M65163). Plasmid pSA10SX was previously constructed as described in YOU *et al.* (1991). It contains the entire *A* gene coding region as well as 1.5 kb of 5′ upstream and 0.4 kb of 3′ flanking DNA. pSA-K is a derivative of pSA10XS and was constructed by site-directed mutagenesis to create a *Kpn*I site at position 884 without any amino acid changes. An inversion within the coding region (pSA-inv1) was created by digesting pSA-K with *Bgl*II (2971) and *Bcl*I (4554), then religating the mixture of fragments and transforming into *Escherichia coli* host JM101. Clones containing the inverted region were identified by restriction enzyme digestion. To delete the *Bcl*I (4554-*Eco*RI (7026) fragment, pSA-K was digested with *Bcl*I then *Eco*RI. The larger fragment was purified from an agarose gel by Elu-Quick and ligated with pUC119. Two site-directed mutations (T4251C and T4254C) were introduced into pSA10XS to construct a plasmid with a unique *Sst*II site (pSA-*Sst*II) without changing the amino acid sequence of the A protein. pSA8.8R which was previously described (FORNEY *et al.* 1983) was digested with *Bgl*II and ligated into pUC119 to create two subclones pSA4.0-5′ (-1034–2971) and pSA4.0-3′ (2971–7026). pSB11.6 is a plasmid that contains the entire *B* gene coding region as well as 5′ and 3′ flanking sequences inserted into pUC119 (SCOTT *et al.* 1993). pSA2.1H (3092–5277) was previously described (YOU *et al.* 1991)

Microinjection: Microinjection of supercoiled plasmid DNA into the d48 macronucleus was performed as previously described by GODISKA *et al.* (1987). Briefly, DNA was dissolved at a concentration of 1–2 mg/ml in either TE buffer (10 mM Tris-HCl, 1 mM EDTA) or microinjection buffer (114 mM KCl, 20 mM NaCl, 3 mM NaH_2PO4, pH 7.4). Approximately 3–6 pl of the DNA solution were injected into the d48 macronucleus using a glass microneedle 1–2-μm diameter at the tip. After microinjection, individual cells were transferred to fresh medium.

Screening pre-autogamy injected cell for transformation: Cell lines were screened to identify transformants using a dot blot procedure obtained from Indiana University (JESSOP-MURRAY *et al.* 1991). Generally, 15–18 fissions after microinjection, about 2500 cells (approximately 400 ng of DNA) were denatured with 0.1 volume of 3 M NaOH at 60° for 30 min, neutralized with 7.5 M NH_4OAc and transferred onto Nytran (Schleicher and Schuell Inc., Keene, New Hampshire). The filter was fixed at 80° for 30 min, then hybridized with the appropriate nick translated probe.

Preparation of paramecium DNA: Approximately 100 ml cultures of paramecium (150,000 cells) were centrifuged and then resuspended in 0.4 ml of their own culture fluid and quickly added to 0.8 ml of lysing solution (1% sodium dodecyl sulfate (SDS), 0.05 M disodium EDTA, 100 mM Tris-HCl, pH 9.5) at 65° for 10 min. The DNA was prepared from lysates by two rounds of phenol-chloroform extraction and ethanol precipitation. The final DNA pellet was resuspended in 20 μl of TE.

Southern blots, nick translations and hybridizations: Southern transfers onto Nytran or nitrocellulose filters were performed according to SAMBROOK *et al.* (1989). Before hybridization, filters were prewashed with 10 × Denhardt solution, 0.1% SDS, 0.2 M phosphate buffer and 5 × SET (1 × SET = 0.15 M NaCl, 30 mM Tris, 2 mM EDTA) at 65° for 1 hr. The filters were then incubated with hybridization solution (1 × Denhardt's solution, 20 mM phosphate buffer, 5 × SET, 0.25% SDS) at 65° for 0.5 hr before adding the labeled probe. After 15–20 hr, filters were washed three times for 30 min each in 0.2 × SET, 0.1% SDS, 0.1% sodium pyrophosphate and 25 mM phosphate buffer at 65° for the first two washes and 70° for the last. The filters were exposed to Kodak X-Omat AR film at -70° with Cronex Lightning-Plus intensifier screens.

Scoring for rescue of d48: In this report transformed cells will refer to cells that stably maintain the injected DNA through vegetative divisions, and rescued cells will refer to lines that have lost the injected DNA but express the A surface protein after formation of a new macronucleus (autogamy). Two methods were used to score cell lines after autogamy. In the daily isolation line method, the injected cell was placed in a depression well and each day a single cell from each depression slide was transferred to a new depression containing 0.8 ml of fresh culture fluid. Autogamy was assessed by staining the starved cell lines with acetocarmine. After autogamy, cell lines were placed at 34° to induce A expression. Lines able to express A after this treatment were considered to have been rescued. To correlate our results with KIM *et al.* (1994) we used another method in which the injected cell was placed in a depression slide, then after three days all the cells were moved to a tube. The tube was alternately fed and starved over a period of 4–6 weeks to induce autogamy. Cells were placed at 34° to induce A expression and scored with anti-A serum.

RESULTS

Regions of the *A* gene that contribute to rescue: Previous studies have established that macronuclear transformation of the d48 mutant with the *A* surface protein gene is sufficient to rescue the A^- mutant phenotype after autogamy (YOU *et al.* 1991; JESSOP-MURRAY 1991; KOIZUMI and KOBAYASHI 1989). The phenotypic rescue of this cell line is the result of correctly incorporating the *A* gene into the next macronucleus. Consistent with previous nomenclature, transformed cell lines will refer to lines containing stable macronuclear copies of the injected plasmid DNA, and rescued cell lines will refer to cells that have formed a new macronucleus and have lost the injected DNA but express the A surface protein according to the anti-A serum test.

To determine the region(s) of the *A* gene that contribute to rescue, several plasmids were constructed that contain fragments of the *A* gene or deletions of internal regions of the gene. These plasmids were microinjected into the macronucleus of d48. Transformed cells were identified by dot blot hybridization before the cells went through autogamy. Transformed and non-transformed (control) cells were cultured in a daily isolation line in

TABLE 1

Frequency of d48 rescue after microinjection of cloned DNA fragments

DNA injected	Pre-autogamy transformed lines	Post-autogamy rescued lines	Percent
pSA10XS	22	18	82
pSA4.0-5′ (-1034-2971)	19	2	11
pSA4.0-3′ (2971-7026)	19	9	47
pSA4.0-5′ + pSA4.0-3′	33	32	97
pSAΔ-1 (deleted 2971-4554)	22	5	23
pSAΔ-2 (deleted 4554-7026)	20	5	25
pSA-inv (inverted 2971-4554)	26	25	96
pSB11.6 (B antigen gene)	17	0	0

which a single cell is moved into a new depression slide each day and the old depressions are allowed to grow until the food is exhausted. Eventually, this results in a depression in which 100% of the cells are in autogamy. After autogamy, cell lines were scored for A expression using anti-A serum. Using this daily isolation line method 100% of the cells from rescued lines expressed the A surface protein and cell lines that were not rescued were 0% A.

The results from transformation with the entire *A* gene (pSA10XS), the 5′ half (pSA4.0-5′) and most of the 3′ portion of the gene (pSA4.0-3′) are shown in Table 1. As expected the entire *A* gene showed a high efficiency of rescue (80%), though not as high as our previous report (100%). This may be a consequence of scoring transformed cell lines by dot blot hybridization rather than A expression. It is possible that a few lines may lose the plasmid before autogamy and in our previous study these lines would not have been included as transformants. You *et al.* (1991) showed that the 8.8-kb *Eco*RI fragment is sufficient to rescue d48. Digestion of this fragment with *Bgl*II results in two nearly equal size fragments that were subcloned (pSA4.0-5′ and pSA4.0-3′) and injected into the macronucleus of d48. The 5′ half has very low activity (1 out of 19), but the 3′ portion rescues about half of the transformed cell lines (9 out of 19). pSA4.0-3′ contains the most divergent region of the *A* gene sequence as compared with related surface protein genes from Paramecium. It also includes a series of tandem repeats 210 bp in length that are >90% identical at the nucleotide level. Results from our colleagues at Indiana University, which were communicated prior to their publication, (see Kim *et al.* 1994) indicated that the tandem repeat region alone was sufficient to rescue d48 though clearly with a lower efficiency than the entire *A* gene. To determine if this was the only region that could rescue d48, the *Bgl*II(2971)-*Bcl*I(4554) fragment that contains the repeats was deleted from the *A* gene (pSAΔ-1, see Figure 1). Table 1 shows that elimination of the repeats results in a significant reduction in rescue, but not a complete loss of activity. We also tested a deletion of a non-overlapping *Bcl*I(4554)-*Eco*RI(7026) fragment (pSAΔ-2). As shown in Table 1 the efficiency of rescue is roughly the same as pSAΔ-1. These results indicate that although the repeats may be sufficient for rescue (Kim *et al.* 1994), other regions of the gene also contribute to rescue activity. The *51B* gene, which is similar to *51A* at the amino acid and nucleotide level, was injected into d48 and 17 high copy number transformants were identified by dot blot analysis. None of the transformants were rescued after autogamy (Table 1). Although this result is not surprising because the d48 mutant has a normal macronuclear copy of the *B* gene, it does illustrate the gene specific effect of rescue and shows that the specificity cannot be overcome by higher than normal copy numbers.

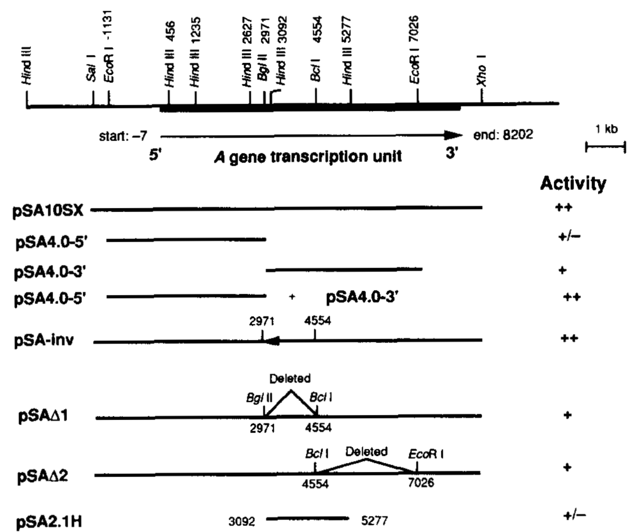

FIGURE 1.—Map of the cloned *A* gene and plasmids used in this report. The *A* transcription unit is shown as a line with an arrow, oriented in the 5′ to 3′ direction as indicated. The stippled box indicates the translated region of the gene. Numbers above the map refer to positions relative to the start of translation. Details on the construction of the plasmids shown are given in MATERIALS AND METHODS. Activity refers to the relative ability of the plasmids to rescue the d48 mutant line after autogamy. Original data are presented in Tables 1 and 2.

The ability of the macronuclear *A* gene to rescue d48 is not understood at the molecular level, but if a specific product (RNA or protein) is encoded by the *A* gene and required for DNA processing, then inversions of sequence spanning those regions should disrupt the product. The *Bgl*II-*Bcl*I fragment (2971-4554) that contains the tandem repeats was inverted within the *A* gene (pSA-inv, see Figure 1). Although deletion of this region resulted in a significant drop in activity, inversion had no measurable effect (25 out of 26 transformants).

The inability to localize 100% of the rescue activity to a defined region suggests that more than one region of the *A* gene may contribute to rescue. To determine if different regions of the *A* gene show cooperativity in the rescue assay, two different portions of the gene, pSA4.0-5′ and pSA4.0-3′ were co-injected into d48. After

TABLE 2

Measurement of d48 rescue using different assays

DNA injected	Autogamy method[a]	Transformed	Rescued	Percent
pSA2.1H	Daily isolation line	18	1	6
pSA2.1H	Tube culture	51	26	51
pSA4.0-5′	Daily isolation line	19	2	11
pSA4.0-5′	Tube culture	19	6	32

[a] See MATERIALS AND METHODS for detailed description.

autogamy 32 out of 33 transformed lines were rescued. Since the pSA4.0-3′ fragment alone rescues less than half the cell lines and the pSA4.0-5′ fragment alone has little activity, this result indicates that there is at least an additive effect when the two fragments are co-injected and perhaps a cooperative effect. This occurs despite the non-contiguous form of the DNA sequences. Both this experiment and the rescue by pSA-inv suggest that only the presence of the DNA in the macronucleus is critical to rescue, not the orientation or form of the DNA.

Different assays for rescue have different sensitivities: Data presented in our previous publication (You *et al.* 1991) showed that a 2.1-kb *Hin*dIII fragment (3092-5277) did not rescue d48. Since this fragment contains the tandem repeats, we became concerned that our earlier data was in error. The experiment was repeated and consistent with our previous results, only one out of 18 transformants was rescued (Table 2, pSA2.1H). One difference between the experiments performed by Kim *et al.* (1994) and ourselves is the handling of cell lines after injection. Instead of generating subclonal lines through a daily isolation line, they typically score a population of cells after autogamy, all of which are derived from a single injected cell (see MATERIALS AND METHODS). Repeating the injection of pSA2.1H using this alternate method resulted in about 30% of the cell lines having some rescued cells although most individual lines were less than 100% A. This is consistent with the data by Kim *et al.* (1994) and indicates that the daily isolation line method is not as sensitive as the large population method. Decreased sensitivity is not inherently a disadvantage since fragments such as pSA4.0-3′ that show about 50% rescue using the daily isolation line method might appear no different than the entire *A* gene if the more sensitive method was used. Because of this difference in sensitivity, the cell lines injected with pSA4.0-5′ were tested using both methods. Both sets of data show that this fragment has very low but detectable rescue activity (Table 2).

The injected DNA does not act as a direct template for the synthesis of the next macronuclear copy of the *A* gene: Since the rescue of d48 requires a copy of the *A* gene in the old macronucleus, one simple model is that the macronuclear copy of the *A* gene acts as a template for the synthesis of the next macronuclear copy of the *A* gene. This was tested directly by transforming d48 with

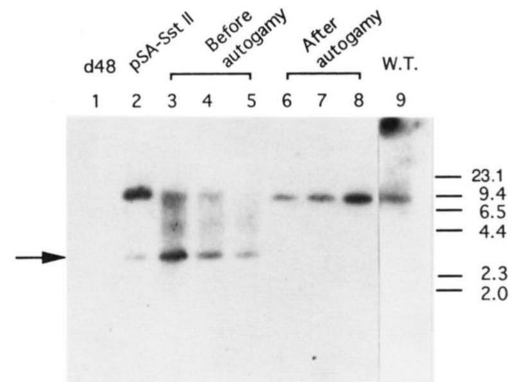

FIGURE 2.—Genomic Southern of d48 injected with pSA-*Sst*II. An *A* gene plasmid with a unique *Sst*II restriction site was injected into d48 cells. Transformants that contain the plasmid were selected and DNA was isolated from these lines before and after autogamy. DNA was digested with *Eco*RI and *Sst*II, electrophoresed on an agarose gel and blotted to nitrocellulose. The filter was probed with pSA8.8R. Lanes: 1, d48; 2, pSA-*Sst*II; 3–5, three transformed lines before autogamy; 6–8, same three cell lines after autogamy; 9, wild-type cells. The arrow indicates the *Eco*RI-*Sst*I fragment that is unique to the injected plasmid and not seen in the macronucleus after autogamy. Size markers are indicated in kilobases.

a copy of the *A* gene that contains a unique *Sst*II restriction site created at position 4251 using site-directed mutagenesis. If the copy of the *A* gene in the old macronucleus is used as a template, then the new macronucleus formed after autogamy should also contain an *Sst*II site. Fifteen out of 17 cell lines that were transformed with pSA-*Sst*II were rescued after autogamy. Figure 2 shows a Southern blot of DNA before and after autogamy from three cell lines. Restriction digests with *Eco*RI and *Sst*II show the DNA before autogamy has a 2.8-kb *Eco*RI-*Sst*II fragment (*lanes 3–5*), but after autogamy this band is not present (*lanes 6–8*). No evidence for an *Sst*II site could be detected in longer exposures, nor was any evidence found for the direct transfer of genetic information to the new macronucleus when the same experiment was performed using a *Kpn*I site engineered at position 884 (data not shown).

DISCUSSION

Previous studies have established that the micronuclei of d48 are normal yet the macronucleus has a deletion of the *A* type variable surface protein gene. As a result of this macronuclear mutation the cell is defective for the ability to express the A surface protein on its surface, and the ability to incorporate the *A* gene into the next macronucleus. Macronuclear transformation with the *A* surface antigen gene can complement both these functions, but it has already been shown that the entire *A* gene is not required to incorporate the *A* gene into the new macronucleus (Jessop-Murray *et al.* 1991; You *et al.* 1991). The present study was initiated to define the sequences within the macronuclear *A* gene that are re-

quired for its correct processing during macronuclear development. The results presented in this paper and KIM *et al.* (1994) are not consistent with a single defined region being responsible for rescue of d48. Although the tandem repeats alone can rescue the cell line, deletion of the repeats does not eliminate rescue activity. Nor does elimination of the repeats have an effect any more severe than removing another non-overlapping region of the 3′ end. If more than one region of the *A* gene can contribute to rescue, then it is possible there are particular primary regions that are critical for rescue and additional accessory regions that enhance the activity. This idea is consistent with the enhanced rescue observed when the 5′ half of the *A* gene (pSA4.0-5′), which has little or no activity, was co-injected with pSA4.0-3′. The gene specific nature of rescue is illustrated by the inability of the *51B* gene or the *P. primaurelia 156G* gene (KIM *et al.* 1994) to rescue the mutant. The *B* gene is 73% and *G* is 79% identical to the *A* gene at the nucleotide level.

We believe models to explain the ability of the *A* gene to rescue d48 can be divided into three categories: (1) the sequences make a product that is required for the incorporation of the *A* gene into the next macronucleus, (2) the *A* gene sequences titrate some other factor or (3) the DNA itself is the required product. Although the first class of models was initially attractive, no evidence for any product made from the A gene sequences, other than the *A* gene mRNA itself, has ever been found (JESSOP-MURRAY *et al.* 1991; J. FORNEY and R. ROLFES, unpublished). Although the inability to find such a product must be interpreted with caution, the results presented here, both the inability to localize the rescue activity to a single region and the inversion of the *BglII-BclI* fragment, cannot be easily explained by a model that proposes a specific product. In other experiments we have introduced frameshifts into the 3′ region of the *A* gene and found that these have no effect on the rescue activity (Y. YOU and J. FORNEY, unpublished).

Models that propose titration of another molecule suffer from the requirement of a different gene specific factor for each locus which demonstrates this type of non-Mendelian inheritance. Thus far, the list of such loci only includes the *A* gene, mating type (SONNEBORN 1977) and a trichocyst non-discharge mutant (SONNEBORN and SCHNELLER 1979), but we have recently been able to construct a non-Mendelian mutant for the *B* locus (J. SCOTT, K. MIKAMI and J. FORNEY, unpublished), and we suspect that many additional genes near macronuclear telomeres are under similar control. One experiment that could be viewed as support for a titration model was the induction of a macronuclear deletion of the *P. primaurelia*, stock *156G* gene by transforming the macronucleus with high copy numbers of the cloned *G* gene (MEYER 1992). We have not been able to induce this effect in *P. tetraurelia* with the *A* gene, nor have we seen any effect of high copy number of *A* gene fragments before autogamy on the rescue of d48 (Y. YOU and J. FORNEY, unpublished). It is difficult to reconcile a titration model with the ability of non-overlapping fragments of the *A* gene to rescue d48. It would require specific binding sites that are present in multiple sites of the *A* gene but not present in the *156G* gene (79% nucleotide sequence identity). More elaborate models that involve transposable elements could be imagined, but two experiments argue against the involvement of transposons. First, KOIZUMI and KOBAYASHI (1989) showed that removal of the d48 micronuclei and replacement with a wild-type micronucleus resulted in a cell line that was still d48. Second, analysis of wild-type and d48 micronuclear DNA by PREER *et al.* (1992) showed that the *A* gene restriction fragments that were probed in both cell lines are identical. Neither result would be expected for a typical transposon.In contrast, all the current experimental data is consistent with more speculative models that propose the macronuclear DNA itself is the required factor and it acts through specific base pair interactions. This would account for: (1) the inability to identify any additional product from the *A* gene, (2) the sequence specificity of d48 rescue and (3) the observation that large DNA fragments generally rescue with higher efficiency than small fragments. At least two possible functions of the DNA during macronuclear development could be envisioned, a role in amplification as gene specific DNA replication primers, or a role in the removal of micronuclear specific DNA sequences. Unfortunately, there is no direct evidence for the transfer of DNA from the old macronucleus into the macronuclear anlagen. BERGER (1974) demonstrated that tritiated thymidine which was originally incorporated into the old macronucleus eventually is incorporated into the new macronuclear anlagen. Although most of the label found in the anlagen must result from the degradation of macronuclear fragments and reincorporation of DNA precursors, this study could not rule out the transfer of small amounts of old macronuclear DNA into the new macronucleus. In the future we hope to devise experiments to specifically address this possibility.

The recent development of a technique to isolate micronuclear DNA from *P. tetraurelia* has led to the discovery that the micronuclear *A* gene contains internal sequences which are eliminated during macronuclear development (PREER *et al.* 1992). These internally eliminated sequences (IESs) are known to occur in other ciliates, but the molecular mechanism of their removal is not well understood. A future line of investigation will focus on the possibility that removal of these sequences is defective in d48.

We thank JOHN PREER, SUE KIM and BARRY POLISKY for discussion of their results prior to publication. This work was supported by the National Institutes of Health grant GM43357, as well as a Junior Faculty

Award from the American Cancer Society to J.F. This is journal paper no. 13889 from the Purdue Agriculture Experiment Station.

LITERATURE CITED

BERGER, J. D., 1974 Selective autolysis of nuclei as a source of DNA precursors in *Paramecium aurelia* exconjugants. J. Protozool. **21:** 145–152.

BLACKBURN, E. H., and K. M. KARRER, 1986 Genomic reorganization in ciliated protozoans. Annu. Rev. Genet. **20:** 501–521.

EPSTEIN, L. M., and J. D. FORNEY, 1984 Mendelian and non-Mendelian mutations affecting surface antigen expression in *Paramecium tetraurelia*. Mol. Cell. Biol. **4:** 1583–1590.

FORNEY, J. D., and E. H. BLACKBURN, 1988 Developmentally controlled telomere addition in wild-type and mutant paramecia. Mol. Cell. Biol. **8:** 251–258.

FORNEY, J. D., L. M. EPSTEIN, L. B. PREER, B. M. RUDMAN, D. J. WIDMAYER *et al.*, 1983 Structure and expression of genes for surface proteins in *Paramecium*. Mol. Cell. Biol. **3:** 466–474.

GODISKA, R., K. J. AUFDERHEIDE, D. GILLEY, P. HENDRIE, T. FITZWATER *et al.*, 1987 Transformation of *Paramecium* by microinjection of a cloned serotype gene. Proc. Natl. Acad. Sci. USA **84:** 7590–7594.

HARUMOTO, T. 1986 Induced change in a non-Mendelian determinant by transplantation of macronucleoplasm in *Paramecium tetraurelia*. Mol. Cell. Biol. **6:** 3498–3501.

JESSOP-MURRAY, H., L. D. MARTIN, D. GILLEY, J. R. PREER, JR., and B. POLISKY, 1991 Permanent rescue of a non-Mendelian mutation of Paramecium by microinjection of specific DNA sequences. Genetics **129:** 727–734.

KIM, C. S., J. R. PREER, JR., and B. POLISKY, 1994 Identification of DNA sequences that control processing of DNA in macronuclear development in *Paramecium*. Genetics **136:** 1325–1328.

KOBAYASHI, S., AND S. KOIZUMI, 1990 Characterization of Mendelian and non-Mendelian strains by micronuclear transplantation in *Paramecium tetraurelia*. J. Protozool. **37:** 489–492.

KOIZUMI, S., and S. KOBAYASHI, 1989 Microinjection of plasmid DNA encoding the A surface antigen of *Paramecium tetraurelia* restores the ability to regenerate a wild-type macronucleus. Mol. Cell. Biol. **9:** 4398–4401.

MEYER, E., 1992 Induction of specific macronuclear developmental mutations by microinjection of a cloned telomeric gene in *Paramecium primaurelia*. Genes Dev. **6:** 211–222.

PREER, L. B., G. HAMILTON and J. R. PREER, JR. 1992 Micronuclear DNA from *Paramecium tetraurelia*: serotype 51 A gene has internally eliminated sequences. J. Protozool. **39:** 678–682.

RUDMAN, B., L. B. PREER, B. POLISKY and J. R. PREER, JR., 1991 Mutants affecting processing of DNA in macronuclear development in *Paramecium*. Genetics **129:** 47–56.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning: A Laboratory Manual*, Ed. 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SCOTT, J., C. LEECK and J. FORNEY, 1993 Molecular and genetic analyses of the B type surface protein gene from *Paramecium tetraurelia*. Genetics **133:** 189–198.

SONNEBORN, T. M., 1950 Methods in the general biology and genetics of *Paramecium aurelia*. J. Exp. Zool. **113:** 87–148.

SONNEBORN, T. M. 1977 Genetics of cellular differentiation: stable nuclear differentiation in eucaryotic unicells. Annu. Rev. Genet. **11:** 349–367.

SONNEBORN, T. M., and M. V. SCHNELLER, 1979 A genetic system for alternative stable characteristics in genomically identical homozygous clones. Dev. Genet. **1:** 21–46.

YAO, M. C., 1989 Site-specific chromosome breakage and DNA deletion in ciliates, pp. 715–734 in *Mobile DNA* edited by D. E. BERG and M. M. HOWE. American Society for Microbiology, Washington, D.C.

YOU, Y., K. AUFDERHEIDE, J. MORAND, K. RODKEY and J. FORNEY, 1991 Macronuclear transformation with specific DNA fragments controls the content of the new macronuclear genome in *Paramecium tetraurelia*. Mol. Cell. Biol. **11:** 1133–1137.

Communicating editor: S. L. ALLEN