

# Induced Change in a Non-Mendelian Determinant by Transplantation of Macronucleoplasm in *Paramecium tetraurelia*

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**Mutant strain d48 of *Paramecium tetraurelia* lacks the gene for antigen A in the macronucleus, whereas this gene is present in the micronucleus. Transfer of macronucleoplasm from the wild type to strain d48 caused d48 to revert to the wild type after autogamy. Transfer of cytoplasm was not as effective as transfer of macronucleoplasm. It was also found that the micronucleus of d48 developed normally when it was transplanted to wild-type cells, whereas the micronucleus of the wild type formed a macronucleus that lacked the antigen A gene when this micronucleus was transplanted into d48. It was concluded that the micronucleus of d48 has a normal antigen A gene and that the hereditary determinants responsible for the d48 trait are located in the macronucleus. Molecular analysis of d48 clones that had been induced to revert to the wild type revealed that they possessed the antigen A gene in the macronucleus.**

Strain d48 of *Paramecium tetraurelia* was isolated by Epstein and Forney (1). It cannot produce the cell surface protein known as immobilization antigen A. Epstein and Forney showed that this defect occurs because the gene encoding this antigen (hereinafter the A gene) is lacking in the macronucleus. They concluded from a genetic analysis that the gene is present in the micronuclei from which new macronuclei are regularly formed at each autogamy and conjugation. The phenotype of d48 is normal in other respects, and it appears that the elimination of the A gene during macronucleus formation is quite specific. Only the A gene and sequences downstream from it appear to be eliminated.

In crosses of d48 to the wild type, the exconjugant F1 and F2 clones usually retain the phenotype of their cytoplasmic parents. Thus, newly forming macronuclei retain the A gene when they develop in wild-type cytoplasm but usually fail to retain the A gene when they develop in d48 cytoplasm. However, this pattern need not indicate that cytoplasmic factors present in the cytoplasm are genetic determinants, for Sonneborn (5) showed that the same pattern is also exhibited by other traits whose determinants lie in the macronucleus. Two examples in *P. tetraurelia* are the inheritance of mating types (5) and of a nondischarge trichocyst mutation described by Sonneborn and Schneller (7). In both of these cases, cytoplasmic factors specified by the old macronucleus determine that the newly forming macronucleus will be like the old. The molecular basis for these systems is completely unknown. In this paper it is shown that the transfer of macronucleoplasm from cells able to produce the A antigen could cause d48 to revert to the wild type after autogamy. These results suggest that, like the other cases just discussed, the hereditary determinants responsible for the d48 trait are located in the macronucleus and that they act by producing cytoplasmic factors that determine the differentiation of the new macronucleus.

## MATERIALS AND METHODS

**Stocks and culture conditions.** Stock cultures used for this work were *Paramecium tetraurelia* 51s (kappa-free), d4-94 *pwA* (pawn), and d48 *tw* (twisty). d48 was obtained from

stock 51 by X-ray mutagenesis (1). This stock fails to exhibit serotype A even when cultured at high temperatures and it lacks the A gene in its macronucleus. d48 is inherited cytoplasmically. Cells were cultured in a 2.5% Cerophyl (Agri-Tech, Kansas City, Mo.) infusion supplemented with 1 mg of stigmasterol per liter, and cultures were inoculated with *Klebsiella pneumoniae* 1 day before use. Cultures were normally kept at 27°C. Antigen A was induced by culturing the cells at 34°C.

For most of the experiments, donor paramecia of serotype A were cultured in depression slides and usually kept at 27°C. Occasionally, if cells changed their serotype, the culture was replaced by other lines of serotype A. Mutant d48 cells to be injected were cultured in daily isolation lines at the maximum fission rate. After injection, each paramecium was isolated into a depression slide and stored at 27°C. After 2 days all cells in each depression were transferred to a tube with additional culture medium. Approximately 15 fissions after injection, the cells reached their maximum population, starved, and underwent autogamy. Autogamy was confirmed by staining a sample of each culture. Subcultures (samples of 25 autogamous paramecia) were then tested for their ability to produce antigen A, by culturing them for approximately 10 more fissions at 34°C and then exposing samples to anti-A serum.

**Identification of phenotypes.** Identification of serotypes was carried out as described by Sonneborn (6) with sera from the collection at Indiana University. Two genetic markers were also used. One was a morphological mutation, twisty, (*tw*), and the other was pawn (*pwA*). The pawn mutant was isolated by C. Kung (4) and is characterized by its inability to produce avoiding reactions. Cells were tested with a solution containing 40 mM KCL, 1 mM CaCl<sub>2</sub>, and 10 mM Tris hydrochloride (pH 7.2).

**Microinjection.** Transplantation of nuclei and the transfer of nucleoplasm and cytoplasm were carried out as described by Koizumi (3), using an American Optical Corp. phase-contrast microscope with a long-working-distance condenser.

**DNA preparation.** Paramecium DNA was isolated by the procedure described by Epstein and Forney (1) and Forney et al. (2) except that (i) packed cells were suspended in 0.5 ml of their own medium and 1 ml of lysing solution was added

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TABLE 1. Results of microinjections

Material transferred and donor strain	Recipient	Total cells	No. of clones derived from injected cells at frequency (%) of induced antigen A cells:			
			40-100	10-39	2-9	0
<b>Macronucleoplasm</b>						
51(A)	d48(tw) <sup>a</sup>	14	5	2	0	7
pw(A) <sup>b</sup>	d48(tw)	17	4	0	0	13
pw(A)	d48(tw) <sup>c</sup>	30	6	3	2	19
d48(tw)	d48(tw)	46	0	0	0	46
<b>Macronuclei</b>						
pw(A)	d48(tw)	13	7	2	0	5
pw(B)	d48(tw)	10	5	2	0	3
Cytoplasm, pw(A)	d48(tw)	31	1	1	0	29
<b>Noninjected</b>						
d48(tw)		181	0	1	0	180
pw(A)		129	129	0	0	0
pw(B)		25	25	0	0	0

<sup>a</sup> d48(tw), Non-antigen A-producing strain with a twisty marker.

<sup>b</sup> pw(A), Serotype A of strain d4-94 with a pawn marker; pw(B), serotype B of strain d4-94 with a pawn marker.

<sup>c</sup> Only group in which injections were made into the macronucleus rather than the cytoplasm.

rapidly, (ii) extracted DNAs were suspended in distilled water, and (iii) extracted DNAs were treated with RNase (5 µg/ml; Bethesda Research Laboratories, Inc.) for 20 min at room temperature and self-digested pronase (1 mg/ml) with 0.5% sodium dodecyl sulfate for 1 h at 37°C. The mixture was extracted with phenol. The phenol was removed by extraction with chloroform-isoamyl alcohol (24:1).

**Nick translation and Southern hybridization.** Nick translation and Southern blot hybridization were done as described by Forney et al. (2) except that (i) hybridization was at 40°C for 17 h and (ii) filters were washed at 73°C.

## RESULTS

**Transfers of macronucleoplasm.** Approximately 10 pl of macronucleoplasm was transferred from donor cells producing antigen A into the cytoplasm of recipient cells incapable of producing antigen A. In most cases the nuclei of the donors were marked with the pawn gene, and the recipients were marked with the twisty gene. Clones derived from the injected cells were treated as described in Materials and Methods. The percentage of serotype A cells was recorded for each of the clones derived from the injected cells. Results are given in Table 1. The first two lines in the table indicate that 11 of a total of 31 clones produced from injected cells were serotype A cells. The antigen A-producing lines, originally from injected cells, were subsequently carried for many fissions, and they remained stable.

In clones arising from 46 control cells injected with nucleoplasm from non-antigen A-producing d48 cells (Table 1, line 4), no serotype A was found. Noninjected d48, however, reverted to producing A antigen at a very low frequency. A total of 181 isolations of d48 cells treated like the injected cells resulted in one positive culture (Table 1, line 8). Moreover, reversion to the ability to produce antigen A was also found on one occasion in the d48 stock lines being carried in daily isolation cultures (see Materials and Methods).

The results of transferring macronucleoplasm into the macronucleus (rather than into the cytoplasm) of 30 recipi-

ents was also determined (Table 1, line 3). These transfers resulted in changes of the d48 character at about the same frequency (11 out of 30) already observed for transfers into the cytoplasm (11 out of 31).

When the pawn donor cells capable of producing antigen A were not injected, but simply isolated and carried through the same protocol as injected cells, all produced antigen A in percentages varying from 40 to 100% (Table 1, lines 9 and 10). Although all of the cells were capable of producing antigen A, as few as 40% in some cultures actually did express antigen A. How can one account for percentages of less than 40 which were often exhibited by clones of injected cells (e. g., Table 1, lines 1 and 3)? One possibility is that the clones often consist of mixtures of cells, some capable of expressing antigen A and others not capable. This explanation for percentages of less than 40 was verified by monitoring a series of subclones started from isolations of autogamous cells rather than subcultures of 25 autogamous cells. Various percentages of clones derived from the isolated cells gave rise to cultures capable of producing antigen A (data not shown). Thus, only some of the progeny of injected cells regained the ability to produce normal macronuclei. The time after injection at which the hereditary basis for the d48 character reverted to normal is unknown. It could have occurred during subsequent vegetative fissions or have been delayed until autogamy. Nevertheless, the ability to produce the A antigen was not acquired until the next autogamy.

**Transfers of macronuclei.** Transfers of macronuclei (Table 1, lines 5 and 6) were also very effective in causing the d48 character to revert to the wild type. In most of the microinjections, the donor cells capable of producing antigen A were serotype A when the injections were made. An attempt was made to see whether the serotype of the donor cells was important in the frequency of induced change; thus, for one series of injections, serotype B donors were used. The results revealed no differences between serotype A donors (line 5) and serotype B donors (line 6).

**Transfers of cytoplasm.** Only 2 of 31 cells injected with cytoplasm reverted to normal antigen A production. Although these numbers were small, injection of cytoplasm appeared to induce reversion at rates above the spontaneous background rate and below the rate for injected nucleoplasm and macronuclei.

**Transfers of micronuclei.** Although no attempts were made to transfer micronuclei, on two occasions the gene markers indicated that micronuclei were indeed transferred. In one experiment a large amount of cytoplasm from non-antigen A-producing strain d48 (twisty) was injected into the cytoplasm of four lines of the antigen A-producing pawn strain to see whether d48 cytoplasm might cause the pawn lines to lose their capacity to produce antigen A. Cytoplasm did not have this effect, however, for all four injected clones resulted in serotype A cells in high percentages. Nevertheless, one clone showed the twisty phenotype, whereas the others showed the pawn phenotype. This result suggests that the micronucleus of d48 twisty was transferred with the cytoplasm and, at the subsequent autogamy, the micronucleus under the influence of the normal antigen A-determining macronucleus produced a normal macronucleus in which the A gene was present.

In the other case macronucleoplasm of the antigen A-producing pawn line was transplanted into the macronucleus of d48 twisty (Table 1, line 3). Of 30 clones, 11 were transformed, but the remaining 19 clones were not serotype A. However, two of the non-serotype A clones contained mixtures of pawn and twisty cells. This result suggests that

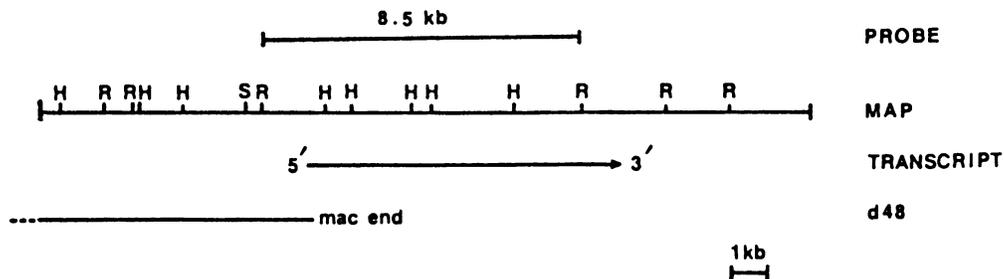


FIG. 1. Restriction map of the cloned A antigen gene and its flanking sequences. The bar above the map represents a fragment used as a probe. The extent of the A antigen transcript is represented by the arrow below the map, and its orientation is indicated. The macronuclear sequences homologous to this region contained in strain d48 are indicated by a bar, and the newly located end of the macronuclear chromosome in this strain is shown. R, *EcoRI*; H, *HindIII*; S, *Sall*.

in each of these two cases the micronucleus of the antigen A-producing pawn line was accidentally transferred to d48. Presumably it found its way into the cytoplasm at the time of injection or subsequent to removal of the microinjection needle. It then persisted during subsequent fissions, and during autogamy under the influence of the d48 macronucleus, it developed new macronuclei that lacked the A gene. Although more cases of micronuclear transfer are needed to draw a firm conclusion, it appeared that even the micronuclei of antigen A-producing cells failed to develop normal macronuclei when transferred to d48 cells.

**Molecular analysis of transformed clones.** Some of the transformed clones and reverted clones were examined by Southern transfers and hybridization to an isolated A gene probe. Whole-cell DNA was extracted, digested with *HindIII*, run on a 1% agarose gel, transferred to a nitrocellulose filter, and hybridized with an 8.5-kilobase (kb) *EcoRI* fragment which contained most of the A gene, as described in Materials and Methods. The restriction map of the A gene and its flanking sequences is shown in Fig. 1. Mutant d48 lacked four bands (2.2 kb, 1.4 kb, 0.7 kb, and 0.5 kb), whereas strain 51 or d4-94 had them (1). Transformed clones and reverted clones showed the same pattern as isolates of strain 51 or d4-94, showing that they had the complete A gene as expected by their phenotype (Fig. 2). The clones in which the micronucleus of d4-94 (pawn) failed to develop normally showed the same pattern as d48. Thus, the A gene was missing in these clones as it was in d48.

## DISCUSSION

These experiments showed that, if a micronucleus from the non-antigen A-producing strain d48 developed at autogamy in cytoplasm that had been injected with an antigen A-producing macronucleus or nucleoplasm from such a nucleus, permanent reversion to an antigen A-producing strain was induced in high frequency. This effect was seen even though autogamy occurred approximately 15 fissions after injection. Whether the injected materials were perpetuated for 15 fissions after injection and then had their effect or whether the hereditary basis for the trait was changed on injection is not clear. In any case, it was found that, when changes to antigen A-producing lines occurred, the phenotype remained non-serotype A until after autogamy, although in some lines the twisty phenotype reverted to normal before autogamy. When transferred into d48, cytoplasm from an antigen A-producing strain appeared to have only weak ability to restore antigen A production. In a comparable fashion it was found in one instance that the accidental transfer of a micronucleus from d48 into an

antigen A-producing strain gave rise to clones descended from the micronucleus of d48 that were normal in their capacity to produce antigen A. Conversely, it was shown in two cases that micronuclei from an antigen A-producing strain, when transferred to d48, lost their ability to produce antigen A. These results, along with our occasional finding of stable reversion (cause unknown) of d48 to the wild type, directly confirmed the conclusions of Epstein and Forney, based on genetic evidence, that the micronucleus of strain d48 contains the A gene and that the hereditary basis for the trait lies outside the micronucleus (1). In addition, these results support the view that the trait is controlled by the old macronucleus via the cytoplasm.

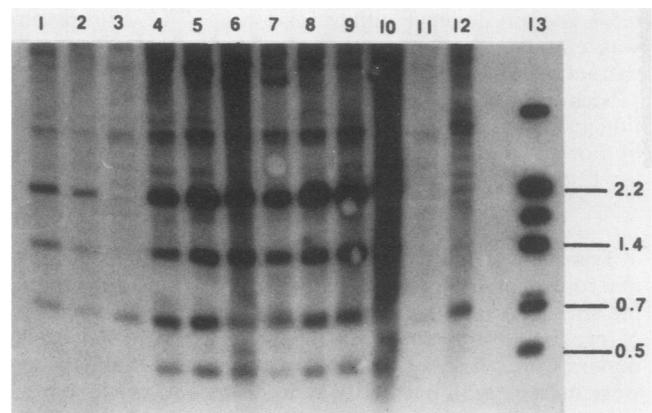


FIG. 2. Hybridization of the 8.5 *EcoRI* fragment to *HindIII*-digested whole-cell DNAs. Since the macronuclei are ca. 800-ploid and the micronuclei are diploid, these preparations consist primarily of macronuclear DNA. All clones show antigen A except those represented in lanes 3, 11, and 12. Lanes: 1, DNA from stock 51; 2, DNA from d4-94 (pawn); 3, DNA from strain d48; 4 and 5, DNAs from reverted clones; 6 through 10, DNAs from transformed clones after autogamy. Lanes 6, 7, and 8 represent clones in which nucleoplasm was transferred from d4-94 (pawn) to d48 macronuclei. Lane 9 represents a clone transformed by the cytoplasm of strain d4-94 (pawn). Lane 10 represents a clone in which a micronucleus of d48 was transplanted into d4-94. Although this lane is overexposed, the original autoradiogram showed the wild-type bands. Lanes 11 and 12 DNAs from clones in which micronuclei of d4-94 (pawn) were transplanted into d48 and underwent subsequent autogamy. These clones did not show serotype A. Lane 13 *HindIII*-digested pSA-8.5 DNA. The pSA-8.5 plasmid, which has been subcloned in the plasmid vector pUC8, contains the 8.5-kb *EcoRI* fragment. The 0.7-kb *HindIII* fragment seen in d48, lanes 11 and 12, represents a cross-hybridizing sequence which comigrates with the 0.7-kb fragment of the A antigen gene in the wild-type digest (1).

It follows that the antigen A-producing macronuclei could ensure the production of a factor (or factors) that acts through the cytoplasm of the developing macronuclear anlagen. This factor would be necessary for proper inclusion of the A gene in the new macronuclei. It would also be necessary for the continued production of the factor. The precise origin and nature of the factor are unknown. The macronuclei of d48 lack not only the A gene but also the capacity to produce the factor.

The inheritance in strain d48 is thus very similar to that of mating types in the group B species of *Paramecium aurelia* (5) and a nondischarge trichocyst trait in *P. tetraurelia* studied by Sonneborn and Schneller (7). In both cases it was shown that the old macronucleus controlled the traits by determining the genetic properties of the developing anlagen. Perhaps in these two cases the alternative characters also resulted from losses of DNA during macronuclear formation.

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