Intra- and Interspecific Complementation of Membrane-inexcitable Mutants of *Paramecium*

NOBUYUKI HAGA, YOSHIRO SAIMI, MIHOKO TAKAHASHI*, and CHING KUNG
Laboratory of Molecular Biology and Department of Genetics, University of Wisconsin, Madison, Wisconsin 53706; and *Institute of Biological Sciences, University of Tsukuba, Ibaraki 305, Japan

**ABSTRACT** Membrane excitation was the basis for backward swimming of *Paramecium* facing stimulus. According to standard genetic tests, inexcitable mutants fell into three complementation groups for both *Paramecium tetraurelia* (*pwA, pwB, and pwC*) and *Paramecium caudatum* (*cnrA, cnrB, and cnrC*). Cytoplasm from a wild type transferred to a mutant through microinjection restored the excitability. Transfusions between genetically defined complementation groups of the same species effected curing, whereas transfusions between different mutants (alleles) of the same group or between sister cells of the same mutant clone did not.

Cytoplasmic transfers of all combinations among the six groups of mutants of the two species showed that any cytoplasm, except those from the same group, was able to cure. Since the pawns and the caudatum nonreversals complement one another through transfusion, they appeared to belong to six different complementation groups. The extent of curing, the amount of transfer needed to cure, and the time course of curing were characteristic of the group that received the transfusion. Variations in these parameters further suggested that the six groups represented six different genes.

Because the donor cytoplasms from either species were equally effective quantitatively in curing a given mutant, the curing factors were not species specific. These factors are discussed.

Ciliated protozoa such as *Paramecium* perform avoiding reactions when stimulated by chemicals, heat, or touch at the anterior (12). An avoiding reaction consists of transient backward swimming that results from the reversal of ciliary-beat direction. Ciliary reversal is due to an increase in intraciliary Ca**, which flows in through the opened calcium channel (21). The voltage-dependent opening of the calcium channel, the essence of membrane excitation, has been investigated in great detail recently using voltage clamps (5, 6, 19).

Some ciliates can be genetically manipulated (22, 31). Mutants defective in their membranes or axonemes and, therefore, in their behavior, have been isolated and characterized (16, 17, 34). Members of one class of behavioral mutants have no avoiding reactions although they are perfectly motile. Such mutants are called pawns (after the chess piece) in *Paramecium tetraurelia* (14, 15), caudatum nonreversal (CNR) in *Paramecium caudatum* (32, 34), and tetrahymena nonreversal in *Tetrahymena pyriformis* (33). Extensive electrophysiological studies show that these mutants fail in generating the calcium-action potential (18, 33, 34). Upon an abrupt depolarization with a voltage clamp, the Ca** inward current in the wild type, which peaks within several ms, is greatly reduced or absent in pawns (24, 25, 28). Complementation in F1 and phenotypic segregation in F2 have led to the conclusion that the more than 200 lines of pawns (representing more than 100 independent mutations) belong to *pwA, pwB, and pwC* (4, 15, 16), and the seven lines of CNR belong to *cnrA, cnrB, and cnrC* (32).

Diffusible substances in the cytoplasm of the wild type can restore excitability in the mutants. This can be achieved naturally through the cytoplasmic bridge during conjugation (1) or artificially through microinjection (7–9, 11). Transferring the cytoplasm of a live wild-type donor or various fractions of a wild-type homogenate to a mutant recipient effects the “cure.” Curing occurs hours after injection, lasts 3–4 d, and corresponds to the recovery of Ca** current upon step depolarization under voltage clamp (7, 8). Such cytoplasmic “transfusion” among many pawns shows that cytoplasmic complementation strictly parallels the genetic complementation so that microinjection can be used to classify the pawns (8).

The question of whether any pawns are genetically equiv-
before and after Cytoplasmic Transfusion barium-solution, indicating restored calcium channel activity, with the cytoplasm of species. Fig. 1 shows that dance hours after receiving the cytoplasm from the other ward steps (17, 20). All inexcitable mutants performed this injection of cytoplasm from a wild type or a mutant of a different complementation group of the same species (7, 8, 31, 32). Paramaecia were cultured in Cerophyl medium (Cerophyl Laboratories, Inc., Kansas City, MO) buffered with sodium phosphates and bacterized with Enterobacter aerogenes (30). Cells were grown at 23°C except in the case of pwC, a heat-sensitive mutant, whose phenotype was best expressed after they were grown at 35°C (2, 3, 26).

Microinjection and Behavioral Test: Koizumi's method of microinjecting paramaecia (13) was modified as described by Haga et al. (8): Approximately 15–20 pl of cytoplasm was injected into a P. tetraurelia and 45–50 pl into a P. caudatum, the larger paramaecia. These volumes, ~12% of the total volume of the recipient, were the largest possible for single injections into live cells. The injected cells were incubated in a resting solution and were periodically withdrawn and transferred to a test solution with a micropipette. The test solution was the Dryr's solution enriched with 20 mM KCl. The duration of the backward swimming induced by the transfer was a quantitative measurement of the excitability of the paramecium (8).

Dark-field Photography and Electrophysiology: A polaroid camera mounted over a glass plate illuminated at a low angle with a Schott KL-1500 light source (Schott Optical Glassware, Inc., Duryea, PA) in a dark-room was used to register the behavior of the injected cells in a test solution. This has been described previously (2). Methods for capturing, rinsing, immobilizing, penetrating, and recording paramaecia have been established (20). The voltage-clamp method was also standard (25). Cells were bathed in 1 mM K+, 1 mM Ca++, 10 mM tetraethylammonium+, 1 mM HEPES, and 10 mM EDTA, pH 7.3, during recording. All chemicals are reagent grade; cations were from chloride salts.

RESULTS

Interspecific Restorations of Membrane Excitability

We have previously shown that the ability of a pawn or a CNR mutant to swim backward can be restored by microinjection of cytoplasm from a wild type or a mutant of a different complementation group of the same species (7, 8, 11). We show here that transfer of cytoplasm between P. tetraurelia and P. caudatum also effects such a "curing" of the mutants. A mutant receiving the foreign cytoplasm, like those receiving potent cytoplasm of the same species, regained its ability to swim backward and have the avoiding reactions when properly stimulated. One standard method for showing the avoiding reactions was to transfer the cells into Ba++-containing solutions. Because of the generation of the all-or-none Ba-Ca action potentials, the excitable cells performed the “Ba-dance” with almost regular rapid backward and forward steps (17, 20). All inexcitable mutants performed this dance hours after receiving the cytoplasm from the other species. Fig. 1 shows that cnrC cells of P. caudatum injected with the cytoplasm of pwC of P. tetraurelia danced in the barium-solution, indicating restored calcium channel activity, whereas control cells (cnrC injected with cnrC cytoplasm) did not dance, showing no restoration.

Electric Properties of the Mutant Membrane before and after Cytoplasmic Transfusion

The avoiding reaction resulted from an action potential and was therefore an indirect indicator of the excitability of the membrane. The membrane excitability can be directly measured with electrodes since paramecium is large enough for convenient intracellular recording (5, 19, 20, 27). The best quantitative analysis of membrane excitability employs a voltage clamp to control the voltage and examine the voltage-dependent ion currents across the membrane. Voltage clamp experiments with wild-type Paramecium show that a step depolarization from the resting level (~40 mV, inside negative) by several tens of millivolts (e.g., to ~15 mV) triggers a transient inward current carried by Ca++ that peaks in several milliseconds and subsides within another 5 ms (5, 19, 24). The peak inward current was ~7 nA in wild-type P. caudatum previously incubated in the resting buffer. Identical experiments with the pawn (24, 28) or the CNR mutants gave no inward current (Fig. 2A). We showed earlier that pawn mutants regain the calcium current hours after a transfection from a wild type or from a mutant of a different complementation group of the same species (8). We report here that the same intraspecific restoration of calcium current was true of the CNR (Fig. 2, B–D). We also found that such a restoration of the calcium current could be achieved by transferring cytoplasm of a different species. For example, as shown in Fig. 2, E–G, cnrC of P. caudatum regained the calcium current after receiving cytoplasm from pwA, pwB, or pwC of P. tetraurelia. The restored currents could be as large as those in the wild type, i.e., ~7 nA. The electrophysiological findings show that the restoration of the avoiding reaction as demonstrated in Fig. 1 was not due to some trivial addition of ions to the recipient but reflected a profound change in the membrane excitability of these mutants.

Complementation Matrix

A quantitative measurement of a paramecium’s membrane excitability is easily obtained by transferring it from a resting buffer to a test solution containing a high concentration of K+ and timing the duration of the first continuous backward swimming induced by this transfer (8). This duration is proportional to the inward calcium current under voltage clamp

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**Figure 2.** Membrane currents (Im) induced by step depolarization (Vm) in different P. caudatum cells. All currents were recorded from cnrC mutants except the top traces of A. The voltage steps are given at the top left and the two current traces from each cell are superposed in the figures. A step from −44 mV to −30 mV or to −15 mV induced a small or a larger current in the excitable cells (5, 24, 25). The peak current induced by the larger voltage step was ~6 nA (range: 4–9 nA) in the uninjected wild type, G3, previously kept in the rest solution for 6–8 h (A, top traces). The same voltage steps did not induce the inward current in the inexcitable mutant cnrC similarly treated (A, bottom traces). cnrC mutants became excitable and generated the inward current 6–8 h after they were injected with ~50 pl of cytoplasm from (B) cnrA, a P. caudatum mutant of a different complementation group; (C) cnrB, a P. caudatum mutant of yet another group; and (E) pwA, (F) pwB, and (G) pwC, mutants of three complementation groups in P. tetraurelia. The restored calcium currents were similar to that of an untreated wild-type paramecium. Injection of the cytoplasm from sister cnrC donors to cnrC recipients did not restore the calcium current in the recipients (D). (Y. Satow and N. Haga, unpublished results) and therefore provides an estimate of the amplitude of the calcium current restored by microinjection. Wild-type P. tetraurelia swam backward for ~45 s and wild-type P. caudatum swam backward for ~60 s in this test. Table 1 summarizes a complete study of the restoration of excitability, thus measured, with all eight cytoplasmic donors (the two wild types and the six mutants) and all six mutant recipients. Uninjected mutants or those injected with cytoplasm of the sister cells of the same clone showed no backward swimming (0 s for pwA, pwB, cnrA, and cnrB) or barely measurable backing (2–3 s for pwC and cnrC). In contrast, all pawns and cnrC showed complete excitability after being injected with other cytoplasm (30–50 s for pwA, pwB, and pwC; 70–75 s for cnrC). With the standard transfusion of 12% recipient cell volume, cnrB was cured to a lesser extent (10–20 s), whereas cnrA was not cured at all.

**Figure 3.** Time course of the restoration of the ability to swim backward after microinjection of the cytoplasm. The recipients were injected at time 0 (arrows), periodically withdrawn from the rest solution, and transferred into Dryl's solution with 20 mM K⁺. The duration of backward swimming induced by this transfer, which is an estimate of the membrane excitability, was clocked and plotted over time. A shows that the cytoplasm of wild type, stock G3 of P. caudatum, injected into cnrC, a mutant of the same species, restored the ability of the mutant to swim backward (O, mean ± SD, n = 6), whereas the cytoplasm from sister cnrC donors did not restore (Δ, n = 6). B shows that the cytoplasm of a wild type, stock 51 of P. tetraurelia, also restored the ability of cnrC, the P. caudatum mutant, to swim backward. The time courses of cnrC curing by the cytoplasms of the two species were very similar. C shows that cytoplasm of G3, twice injected into cnrA, a different mutant of P. caudatum, also restored the ability of this mutant to swim backward (O, n = 10), whereas the cytoplasm of sister cnrA cells did not restore the ability (Δ, n = 6). Note that the restored activity in cnrA was smaller and lasted shorter than in cnrC (see text).
However, a second injection, 1 h after the first to allow for recuperation, can partially restore the ability to swim backward even in cnrA (see Fig. 3C).

**Time Courses of Curing**

Curing the inexcitable mutants with foreign cytoplasm took time. Upon periodic testing, we showed previously that pawns, for example, first regain detectable backward swimming by ~2 h after injection. Complete restoration occurs by 6–8 h. Full excitability is maintained for 1 d after which it gradually subsides until it is barely measurable after 3–4 d (8). The time course of the rise and fall of excitability after injection in cnrC was similar to those of the pawns (Fig. 3, A and B). The curing time course, like the extent of curing, was characteristic of the recipients and not the donors, even though the donors may be of two different species, e.g., the time course of curing of cnrC with G3 cytoplasm (Fig. 3A) was the same as that with 51s cytoplasm (Fig. 3B).

The course of curing of cnrB was similar to that of pawns and cnrC, although the extent of curing was smaller (data not shown). The course of curing of cnrA, however, was different. After two injections spaced 1 h apart, the excitability, as measured by the K⁺-induced backward swimming, gradually rose in the first 6 h as in other mutants. However, the excitability was not maintained for days. The bulk of the restored excitability was lost by 10 h after the second injection (Fig. 3C).

**DISCUSSION**

Since the inexcitable mutants could be temporarily cured by transfusions of heterologous cytoplasms, these cytoplasms contained the gene products missing or defective in the mutants. Our previous study (8) shows that mutual curing among the pawn mutants through microinjection separates them into groups exactly as defined by genetic complementation. Thus, as long as a donor has the normal product of a particular gene, it is capable of curing a recipient that has a mutation in that gene. The normal gene product can be provided by the wild type or by mutants defective in different genes. The effectiveness and efficiency of curing were clearly functions of the recipients (compare columns in Table 1 and Fig. 3, A and B with 3 C) and not the donors (compare entries in each column in Table 1 and Fig. 3A and 3B). Because we found that cytoplasms from a different species could cure the mutants to the same extent and with the same time course, the normal gene products from the two species of *Paramecium* appeared to be functionally equivalent.

The curing was not due to the transfer of nuclear fragments because the nuclei were anchored and we were careful to place the micropipette away from the nuclei. In theory, the gene products transferred can be from either RNA (23) or protein (7, 8). In our experiments, curing of the pawns and cnrC could take place in the absence of translation. This, and other experiments with the fractions active in curing, demonstrated that these molecules are proteinaceous. Fractionations of the proteins that effect the cure in the mutants are in progress.

The finding that all pawns complement all CNR is very interesting. The simplest interpretation of these results is that these mutants defined six different complementation groups and therefore six different functional genes. This interpretation rests heavily on observing pawns, where extensive studies show that no curing of a recipient (cytoplasmic complementation) ever results from injection of a cytoplasm from a donor mutant previously defined by breeding analysis to be of the same complementation group. In other words, intragenic complementation has never been observed in pawns by either the genetic or the cytoplasmic tests. Formally, in the absence of intragenic complementation, a new complementation mutation defines a new gene. Since the number of alleles in each cnr group remained small, the interpretation that there were at least six genes involved in the excitability phenotype is not entirely secure. The possibility of intragenic complementation among different alleles of each of the cnr has not been completely ruled out. Nonetheless, two alleles of cnrB did not complement according to our microinjection tests and two alleles of cnrA also did not complement, even after double injections (N. Haga, unpublished results). Although the above formal reservation remains, there were other indications that the CNR harbored mutations in genes different from those of pawns. cnrA was most difficult to cure and the curing only lasted a short time. cnrB was significantly

**Table 1**

| Curing Matrix Showing the Duration of Backward Swimming in the K⁺-test Solution after Injection* |
|---------------------------------------------------------------|
| **Wild type** | **P. tetraurelia** | **P. caudatum** |
|----------------|------------------|----------------|
| **P. tetraurelia** | **pwA** | **pwB** | **pwC** | **cnrA** | **cnrB** | **cnrC** |
| 34.4 ± 12.8 (8) | 41.0 ± 5.2 (7) | 50.8 ± 5.8 (6) | 12.0 ± 1.4 (3) | 12.2 ± 2.8 (6) | 71.0 ± 5.5 (6) |
| 0 ± 0 (47) | 51.5 ± 18.3 (8) | 41.5 ± 18.3 (8) | 19.0 ± 1.4 (3) | 15.0 ± 2.0 (6) | 70.3 ± 9.4 (6) |
| 39.0 ± 12.7 (12) | 0 ± 0 (17) | 36.0 ± 6.6 (6) | 29.0 ± 2.6 (3) | 17.7 ± 3.4 (6) | 76.2 ± 12.2 (6) |
| 35.0 ± 12.4 (6) | 35.7 ± 12.0 (6) | 3.0 ± 3.6 (11) | 26.0 ± 6.1 (3) | 11.7 ± 3.4 (6) | 71.7 ± 11.1 (6) |
| **P. caudatum** | **cnrA** | **cnrB** | **cnrC** |
| 34.0 ± 3.7 (6) | 47.3 ± 5.4 (6) | 51.0 ± 4.4 (6) | 14.7 ± 8.7 (10) | 8.7 ± 3.4 (6) | 76.1 ± 6.4 (6) |
| 37.2 ± 5.2 (6) | 37.0 ± 2.4 (6) | 50.7 ± 5.4 (7) | 0 ± 0 (15) | 15.8 ± 4.6 (6) | 70.8 ± 8.3 (6) |
| 50.3 ± 11.7 (6) | 37.0 ± 4.7 (6) | 38.7 ± 4.1 (6) | 61.5 ± 19.1 (2) | 0 ± 0 (6) | 69.2 ± 7.6 (6) |
| 36.8 ± 6.6 (6) | 47.5 ± 8.9 (6) | 47.8 ± 7.7 (6) | 52.5 ± 3.5 (2) | 19.2 ± 8.8 (6) | 2.7 ± 2.3 (6) |
| **No injection** | **0 ± 0 (10)** | **0 ± 0 (10)** | **2.4 ± 2.7 (6)** | **0 ± 0 (10)** | **0 ± 0 (10)** | **1.8 ± 1.8 (10)** |

* Values are in seconds, mean ± SD (n); uninjected wild-type *P. tetraurelia* (51s): 45 ± 3.8 (10); uninjected wild-type *P. caudatum* (G3): 60.2 ± 4.8 (10). Cells were injected with ~12% of their volume and tested ~6 h after injection.

* *P. tetraurelia* and *P. caudatum* acting as recipients.

* Double injections were spaced 1 h apart. See Fig. 3C and text.

* *P. tetraurelia* and *P. caudatum* acting as donors.
more difficult to cure than the pawns (Table 1, Fig. 3), which may be due to certain inherent properties of cnrB that made it less receptive to the curing factor. Finally, cnrC is clearly a mutant of a different sort since the factor that cured it has now been traced to a postmicrosomal soluble fraction and is therefore most likely a soluble protein, whereas the factors that cured the three pawns have been traced to a microsomal pellet and are probably proteins bound to internal membranes (7, 9).

That pwA, pwB, pwC, cnrA, cnrB, and cnrC are likely to be six different genes in both species is an unexpected result. Over 100 independent mutational events leading to mutations in pwA and pwB have been analyzed (4, 16). Although relatively rare, more than five independent mutations of pwC have been observed. It is therefore surprising that we have not yet encountered mutations in the counterparts of cnrA, cnrB, and cnrC in P. tetraurelia. Although not as many mutants have been analyzed, it is equally surprising that the counterparts of pwA and pwB mutants have not been found in P. caudatum since they appear to be abundant, at least in the other species. One possible explanation may be that the methods employed to select pawns (4, 15, 29) and CNR (32) are different in actual execution, although based on the same principle. If pawns and CNR are different mutants, there is hope of finding all six kinds of mutants in both species simply by using different mutagens and/or different mutant-screening methods. Progress is being made toward identifying the factors in the cytoplasm that effect the cure and comparing the factors isolated from the two species. It would be interesting to find out whether these proteins act directly or indirectly (e.g., catalyze a reaction) to restore the calcium-channel activity.

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REFERENCES

1. Berger, J. D. 1976. Gene expression and phenotypic change in Paramecium tetraurelia excconjugants. Genet. Res. 27:123–134.
2. Chang, S. Y., and C. Kung. 1973. Temperature-sensitive pawns: conditional behavioral mutants of Paramecium aurelia. Science (Wash. DC). 180:1197–1199.
3. Chang, S. Y., and C. Kung. 1973. Genetic analysis of heat-sensitive pawn mutants of Paramecium aurelia. Genetics. 75:49–59.
4. Chang, S. Y., J. Van Houten, L. Robles, S. Lui, and C. Kung. 1974. An extensive study of membrane currents in Paramecium aurelia. Proc. Natl. Acad. Sci. USA. 71:2703–2706.
5. Eckert, R. 1972. Genetic modifications of electric properties in an excitable membrane. Proc. Natl. Acad. Sci. USA. 69:93–97.
6. Haga, N., M. A. Forte, Y. Saimi, and C. Kung. 1981. Characterization and partial purification of a factor controlling the generation of action potential in Paramecium. In Society for Neuroscience 11th Annual Meeting Abstracts. Society for Neuroscience, Bethesda, Maryland. 15a. (Abstr.)
7. Haga, N., M. Forte, Y. Saimi, and C. Kung. 1982. Microinjection of cytoplasm as a test of reintegration in Paramecium. J. Cell Biol. 93:898–904.
8. Haga, N., M. Forte, Y. Saimi, and C. Kung. 1982. Microinjection of wild-type cytoplasm.
9. 41. Naitoh, Y., and K. Hiwatashi. 1982. Soluble gene product controlling membrane excitability in Paramecium. Cell Biology International Report 6:295–300.
10. Hiwatashi, K. 1979. Paramecium. In In VitroFertilization II. C. B. Mertz and A. Monroy, editors. Academic Press, Inc., New York. 255–293.
11. Hiwatashi, K., N. Haga, and M. Takahashi. 1980. Restoration of membrane excitability in a behavioral mutant of Paramecium. Proc. Natl. Acad. Sci. USA. 77:476–480.
12. Jennings, H. S. 1906. Behavior of Lower Organisms. Indiana University Press, Bloomington, Indiana.
13. Kosumi, S. 1974. Microinjection and transfer of cytoplasm in Paramecium. Exp. Cell Res. 88:74–78.
14. Kung, C. 1973. Genetic mutations with altered system of excitation in Paramecium aurelia. I. Phenotypes of the behavioural mutants. Z. vergl. Physiol. 71:142–164.
15. Kung, C. 1973. Genetic mutations with altered system of excitation in Paramecium caudatum II. Mutagenesis, screening and genetic analysis of the mutants. Genetics. 69:29–45.
16. Kung, C. 1979. The biology and genetics of paramecium behavior. In Topics in Neurogenetics. X. O. Benikfield, editor. Elsevier/North Holland Biomedical Press, New York. 1–26.
17. Kung, C., S. Y. Chang, Y. Satow, J. Van Houten, and H. Hansma. 1975. Genetic selection of behavior in Paramecium. Science (Wash. DC). 188:898–904.
18. Kung, C., and R. Eckert. 1972. Genetic modifications of electric properties in an excitable membrane. Proc. Natl. Acad. Sci. USA. 69:93–97.
19. Kung, C., and Y. Saimi. 1982. The physiological basis of taxes in Paramecium. Annu. Rev. Physiol. 44:519–534.
20. Naitoh, Y., and R. Eckert. 1972. Electrophysiology of ciliate protozoa. Exp. Physiol. Biochem. 5:173–339.
21. Naitoh, Y., and H. Kaneko. 1972. Reactivated triton-extracted models of Paramecium tetraurelia Pawn C. Proc. Natl. Acad. Sci. USA. 69:120–124.
22. Naitoh, Y., and H. Kaneko. 1972. Reactivated triton-extracted models of Paramecium tetraurelia Pawn C. Proc. Natl. Acad. Sci. USA. 69:120–124.
23. Naitoh, Y., and R. Eckert. 1972. Electrophysiology of ciliate protozoa. Exp. Physiol. Biochem. 5:173–339.
24. Naitoh, Y., and R. Eckert. 1972. Electrophysiology of ciliate protozoa. Exp. Physiol. Biochem. 5:173–339.
25. Naitoh, Y., and R. Eckert. 1972. Electrophysiology of ciliate protozoa. Exp. Physiol. Biochem. 5:173–339.
26. Naitoh, Y., and H. Kaneko. 1972. Reactivated triton-extracted models of Paramecium tetraurelia Pawn C. Proc. Natl. Acad. Sci. USA. 69:120–124.
27. Naitoh, Y., and H. Kaneko. 1972. Reactivated triton-extracted models of Paramecium tetraurelia Pawn C. Proc. Natl. Acad. Sci. USA. 69:120–124.
28. Naitoh, Y., and H. Kaneko. 1972. Reactivated triton-extracted models of Paramecium tetraurelia Pawn C. Proc. Natl. Acad. Sci. USA. 69:120–124.
29. Naitoh, Y., and H. Kaneko. 1972. Reactivated triton-extracted models of Paramecium tetraurelia Pawn C. Proc. Natl. Acad. Sci. USA. 69:120–124.
30. Naitoh, Y., and H. Kaneko. 1972. Reactivated triton-extracted models of Paramecium tetraurelia Pawn C. Proc. Natl. Acad. Sci. USA. 69:120–124.
31. Naitoh, Y., and H. Kaneko. 1972. Reactivated triton-extracted models of Paramecium tetraurelia Pawn C. Proc. Natl. Acad. Sci. USA. 69:120–124.
32. Naitoh, Y., and H. Kaneko. 1972. Reactivated triton-extracted models of Paramecium tetraurelia Pawn C. Proc. Natl. Acad. Sci. USA. 69:120–124.