GENOMIC EXCLUSION IN TETRAHYMENA[1]

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ABERRANT genetic ratios were observed in crosses of clones of certain inbred strains of *Tetrahymena pyriformis*, syngen 1, first with C* (ALLEN 1960, 1963), then with clones of other strains (NANNEY 1963). In these crosses genes from C*, or from one of these other clones, did not appear in the progeny; hence, the phenomenon was referred to as "genomic exclusion".

The cytogenetic basis of genomic exclusion was worked out using C* and a normal clone from strain AB (ALLEN 1965, 1967a, b). First of all, C* cells were found to have a defective micronucleus, and, in conjugation with AB cells, all the C* meiotic products disintegrated. Secondly, two consecutive rounds of conjugation were discovered to occur invariably following unstopped matings of AB and C*. During the first round, the AB conjugant underwent meiosis normally, generating sister haploid nuclei, the male and female pronuclei. The male pronucleus migrated to the C* conjugant. Then, each pronucleus became diploid, probably by endoreduplication. In each conjugant the diploid syncaryon divided twice mitotically giving rise to two new macronuclei and two new micronuclei, and the old macronucleus started to resorb. However, once the exconjugants separated, the old macronucleus was retained and the new macronuclei were resorbed. The progeny of Round 1 were, therefore, heterocaryons, containing a micronucleus, homozygous in genotype, and a functional old macronucleus, which was genotypically distinct from the micronucleus. Because the old macronucleus was retained, these progeny were sexually mature, unlike the products of normal conjugation in syngen 1. Thus, remating occurred immediately after completion of Round 1. The second conjugation, Round 2, was completely normal and led to the formation of a functional new macronucleus, which was similar in genotype to the micronucleus. Round 2 exconjugants were sexually immature.

The cytological observations established the nuclear basis for genomic exclusion in the AB × C* cross. How general is this nuclear sequence? Does it apply to other cases of genomic exclusion which have been observed in crosses of clones of other strains of syngen 1? In other words do all cases of genomic exclusion have a similar nuclear basis? And, is there a "syndrome" of associated events that leads to genomic exclusion?

From the behavior of the nuclei in the AB × C* cross, a causal sequence of events could be extrapolated. As a prerequisite for genomic exclusion, one of the parents in the cross should have a defective micronucleus. Such a cross should

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show evidence for the two rounds of conjugation, and pairs formed early should give rise to exconjugants that are sexually mature. When remated, the progeny of these crosses should be immature and show aberrant genetic ratios. One should, therefore, observe positive correlations between the presence of defective micronucleate parents, mature progeny, and genomic exclusion in the same crosses. The purpose of this paper is to report data which support this causal sequence of events as occurring more generally in certain crosses within syngen 1, and which show that the genomic exclusion "syndrome" has an hereditary basis.

MATERIALS AND METHODS

Materials: Clones from seven different inbred strains were used in crosses. The strains and their origins are listed below:

Inbred strain	Origin
A	WH-6(I) × WH-14(II)
A1	A-5533 × B-4534
A3	A-11613 × C-11612a
B	WH-6(I) × WH-14(II)
C	UM-226(VI) × B-3537
C1	A-11613 × C-11612a
E	IL-12(VI) × B-9585

In some experiments crosses were made between AB-7a, a clone of the heterozygous AB strain (derived from a cross of A-11613 and B-12614d), and C* (C-5573). AB cells have a normal diploid micronucleus, while C* cells have defective micronuclei or are amicronucleate. The clone designation, A-11 61 3, refers to the A strain, 11th generation of inbreeding, inbred in 1961, and mating type III. In some cases a letter follows the mating type designation and distinguishes clones of different origin.

Crossing techniques: All operations were carried out at 23°C. In some crosses parental cultures grown in Cerophyl-Aerobacter medium (0.15% Cerophyl rye grass infusion with a loopful of *Aerobacter aerogenes*, grown overnight at 30°C), were fed and mixed. Pairs usually formed by 12 hours, but single pairs were not isolated into separate depressions until 48 hours after making the mating mixtures. In other crosses the parental cultures were washed in Dryl's salts solution (DRYL 1959), and timed matings were made (see ALLEN 1967b for details). When washed cultures were mixed, mating occurred after 1 to 2 hours. Single pairs were isolated into depressions containing Cerophyl-Aerobacter at specific times after mating began, usually within 12 hours. Except where indicated, in most crosses the two exconjugants were not separated.

After 3 to 4 days all cultures were examined and could be classified into three types: dead, mature and immature. Cultures were classified as dead if the depression contained less than about ten cells. Mature cultures contained pairs and reacted sexually with samples of a tester culture of nonparental mating type in a "maturity test". Mature cultures contained either Round 1 exconjugants or "nonconjugants", that is, parental cells which prematurely separated without undergoing conjugation. Immature cultures, on the other hand, did not contain pairs and were sexually unreactive when this test was performed. Such cultures arose either as a result of normal conjugation or upon the completion of Round 2 of genomic exclusion. The viability of *any* cross is expressed as the frequency of *immature* cultures.

In some crosses some of the cultures (either mature or immature, or both) contained a variable number of "crinkled" cells, which are amicronucleate. Other cells in these cultures appeared morphologically normal. These mixed cultures will be referred to as "semi-amicronucleate", a term introduced by NANNEY (1957). By this definition, C* is an example of a semi-amicronucleate clone.

In some crosses samples of mature and immature cultures were tested for serotype. The method followed was essentially that described by NANNEY and DUBERT (1960).

Cytological methods: Cultures used for cytological work were grown in 1% proteose-peptone at 23°C. The cells were concentrated by centrifugation, washed in Dryl's physiological salt solution and processed as described previously (ALLEN 1967b), using Gomori's haematoxylin as a nuclear stain (MELANDER and WINGSTRAND 1953).

RESULTS

Semi-amicronucleate clones: Semi-amicronucleate clones are mixed populations of cells containing morphologically normal cells as well as crinkled cells, which could be shown to be amicronucleate, when a nuclear stain was applied. We agree with NANNEY (1957) that amicronucleate cells arise from normal cells by misplacement of all or part of the micronuclear spindle in relation to cytoplasmic cleavage. We agree that crinkled cells cannot initiate new cultures, but we do *not* agree either with NANNEY (1957) or WELLS (1961) that the amicronucleate cells in these mixed cultures *usually* can divide, even once. Observations on dividing cells must be made under oil immersion, since it is difficult to detect chromatin in defective cells during division without high magnification. Under these conditions none of the cells in division were observed to be amicronucleate. Like NANNEY (1957), we also observed large micronuclei or one or more small micronuclei in the morphologically normal cells, but, in addition, we found that small, unaggregated chromosomes could be visualized in dividing, defective cells (ALLEN 1967a) and that these chromosomes showed abnormalities similar to those reported by WELLS (1961). The properties of C* subclones with defective nuclei will be discussed in more detail elsewhere (ALLEN, KOCH and PATRICK 1967).

Although specific mention was not made of the original micronuclear condition of some of the wild strains of syngen 1, apparently both WH-6(I) and WH-14(II) contained a low percentage of amicronucleate cells (ELLIOTT and HAYES 1953), cytological irregularities were noted during conjugation (NANNEY and CAUGHEY 1953), and the viability of the cross was low (NANNEY, CAUGHEY and TEFANKJIAN 1955). Upon crossing, the micronuclear abnormalities disappeared (NANNEY, CAUGHEY and TEFANKJIAN 1955), but during inbreeding they reappeared, and after several generations the frequency of semi-amicronucleate clones became very high in some lineages (NANNEY 1957).

There appear to be three types of semi-amicronucleate clones, two of which have been described previously (NANNEY 1957, 1959). The type first described appeared 40 to 100 fissions after conjugation and was under polygenic control (NANNEY 1957). The second type was delayed in appearance and was found in serially propagated lineages between 500 and 1500 fissions after conjugation. This type seemed to be similar in behavior to the earlier appearing type, although no breeding analysis was carried out (NANNEY 1959).

We wish to report a third type of semi-amicronucleate clone. This type occurred before the 13th fission. It was rarely found during the late generations of inbreeding of strains A, A1, B and C1, but very frequently during the inbreeding of the A3 and C strains, particularly in recent generations.

A record was kept of the frequency of new semi-amicronucleate cultures in

TABLE 1

Frequency of semi-amicronucleate cultures at each transfer of serially propagated lines of inbred C strain

	Transfer							
	1	2	3	4	5	6	7	8
Total isolated	99	305†	278	261	247	240	237	229
No. mature	28*	. .	. .	. .	. .	. .	. .	. .
No. dead	10	27	17	14	7	3	8	2
No. semi-amicronucleate	8	33	37	23	12	25	27	13
Percent semi-amicronucleate	13.1	11.9	14.1	9.3	5.0	11.0	11.8	5.7

* 4/28 semi-amicronucleate (14.3%).
† Five lines from 61 immature, 1st depression cultures were initiated.

serial isolation lines in the 16th generations of inbreeding of the C strain (Table 1). One, or both, of the parents was semi-amicronucleate, and even though morphologically normal subclones were used in making the cross, 28 of the 99 pairs isolated gave rise to mature cultures, four of which were semi-amicronucleate. The frequency of new semi-amicronucleate cultures at each transfer varied from 5 to 13.1%, the mean percentage per transfer being 10.2%. After the 8th transfer only lines that appeared to be morphologically normal were saved; yet, most, when crossed, gave rise to some mature and some immature cultures that were semi-amicronucleate. The early appearance of the semi-amicronucleate condition in strains A3 and C probably explains why both strains have low viability and are in the process of becoming extinct. This very early type of semi-amicronucleate would appear to be a manifestation of an extreme form of precocious senility.

Semi-amicronucleate parents, mature progeny and genomic exclusion: Is there a correlation between the incidence of semi-amicronucleate clones, mature progeny and genomic exclusion? Data on this question, or on portions of this question, were derived from several different sources: (a) from crosses of Round 1 exconjugants derived from AB × C*, the discussion of which will be delayed until the next section; (b) from inbreeding data; and (c) from outcrosses of certain clones of inbred strains not directly related to AB and C*. From the data on inbred strains only information on the correlation between semi-amicronucleate parents and mature progeny could be extracted; only in the outcrosses could the correlation with genomic exclusion also be examined.

The data on the inbred strains suggested that the frequency of semi-amicronucleate clones was strain dependent and that there was a correlation between the frequency of semi-amicronucleate parents and the frequency of mature cultures in their progeny. It should be pointed out that some of these mature cultures probably arose from "nonconjugation" of parental cells. A distinction between cultures containing mature exconjugants and nonconjugants can be made if the micronucleus is examined cytologically, but such an analysis was not made here. For this reason, these cultures will be referred to unambiguously as "mature cultures". It should also be pointed out that crosses were made in the

Cerophyl-Aerobacter medium with pairs being isolated 48 hours after making the mating mixture, except for the most recent generation of inbreeding. This procedure had been adopted because it had been observed that the viability of pairs isolated "late" was higher than those isolated "early". Timed matings in Dryl's salts solution were employed during the most recent generation of inbreeding, and in these crosses the pairs were isolated within 12 hours.

Certain inbred strains (A, A1, B and C1) rarely gave rise to semi-amicronucleate progeny, at least within the first 13 fissions (Table 2). The viability (percent immature cultures) was high in these strains and did not seem to be any lower if timed matings were employed. In these strains the frequency of mature cultures was low; for example, in the most inbred generation the frequencies ran 9.2% (A), 3.3% (A1), 4.7% (B), and 1.6% (C1). A disproportionate contribution to these frequencies was derived from four crosses that included semi-amicronucleate parents. Compared to the average frequency for all

TABLE 2

Viability of inbred strains

Strain	Generation	Mature (%)	Dead (%)	Immature (%)	Total No. pairs isolated	No. crosses	Fraction of crosses with semi-amics	% mature in crosses with semi-amics
A	13	5.1	14.9	80	295	10	....	...
	14	14.7	7.3	78	510	17	....	...
	15*	9.2	7.7	83	480	16	2/16	53.5
A1	13	2.1	0.9	97	300	10	....	...
	14	11.8	5.2	83	540	18	....	...
	15*	3.3	8.7	88	450	15	1/15	23.3
B	14	2.1	3.9	94	288	10	....	...
	15	4.3	21.7	74	465	16	....	...
	16*	4.7	6.3	89	558	19	1/19	63.4
C1	5	2.3	11.7	86	300	10	....	...
	6	11.0	9.0	80	390	13	....	...
	7	21.0	16.0	63	305	11	....	...
	8	7.1	9.9	83	425	15	....	...
	9*	1.6	11.4	87	446	16	0/16	...
A3	5	21.5	26.5	52	297	10	....	...
	6	8.9	15.1	76	360	12	....	...
	7	23.8	26.2	50	450	15	....	...
	8*	29.9	41.1	29	540	18	15/18	32.3
C	13	10.2	43.8	46	420	14	....	...
	14	26.7	31.3	42	360	12	....	...
	15	24.4	33.6	42	657	22	20/22	25.1
	16*	31.0	37.0	32	938	32	26/32	34.3
	16G	23.7	43.5	32.8	494	17	11/17	27.5
	16B†	39.3	29.3	31.4	444	15	15/15	39.3

* Pairs isolated 12 hours after mating in Dryl's salt solution. In earlier generations the matings occurred in Cerophyl-Aerobacter and pairs were isolated about 36 hours after mating began.

† 16B; one or more of four sister lines were semi-amicronucleate. 16G; all five sister lines were normal.

crosses of strains A, A1 and B, the frequency of mature cultures was much higher in these selected crosses (53.5%, 23.3%, 63.4%).

Crosses of the A3 and C strains frequently included semi-amicronucleate clones and gave rise to some progeny which were also semi-amicronucleate. In these crosses the frequency of mature cultures was somewhat higher than the average for the strain. However, so many of the clones were semi-amicronucleate, particularly in the most inbred generation, that the average frequency of mature cultures was almost as high as in the selected group of crosses. Even when rigorous selection for "normal" clones was made in the 16th generation of the C strain (16G), this frequency was still fairly high (23.7%), but significantly lower than in crosses of the "16B" group (39.3%). However, of the so-called "normal" group of 16G clones, only 6/17 of the crosses gave rise to no semi-amicronucleate progeny by 13 fissions. In these six crosses the frequency of mature cultures was 11%, lower than that of other groups of C strain clones. Thus, in the C strain crosses a significant correlation was observed between the severity of the semi-amicronucleate condition in the parental clones and the frequency of mature cultures.

The frequency of mature cultures was also higher in the most inbred generation of the A3 and C strain, when timed matings were employed. If the mature cultures are exconjugant cultures, they would be equivalent to those of Round 1

TABLE 3

Dependence of viability of C strain crosses on time of isolation

	Pairs isolated at 12 hours				Pairs isolated at 36 hours				
Clones crossed	No. mature	No. dead	No. immature	Percent viable	No. mature	No. dead	No. immature	Percent viable	% increase in viability due to genomic exclusion
3dxe	20	8	2	7	5	12	13	43	84
3axe	18	6	6	20	3	17	10	30	33
17axd	14	8	5	18	8	13	7	24	25
26bxd	14	6	10	30	1	13	14	50	40
34axb	13	11	6	20	0	19	11	37	46
34dxe	9	20	1	3	1	25	4	13	77
53cxd	9	10	7	27	0	19	11	37	27
53axb	10	4	16	53	2	3	25	83	36
53dxe	8	7	14	47	2	10	18	60	22
66axb	22	5	3	10	5	14	11	37	73
66dxe	10	2	18	60	2	2	8	67	10
66cxe	9	5	16	53	3	6	21	70	30
70axd	23	4	3	10	12	8	10	33	70
71bxe	12	2	16	53	2	3	25	83	36
71cxe	20	4	4	14	0	7	23	77	82
79dxe	28	2	0	0	13	15	2	7	100
Totals	239	104	127	..	59	186	213	..	..
Percent	50.8	22.1	27.1	..	12.9	40.6	46.5	..	41.7

* *Percent increase in viability due to genomic exclusion* = (% *viable at 36 hr* — % *viable at 12 hrs*)/(% *viable at 36 hrs.*)

exconjugants and their frequency should depend upon the time at which pairs are isolated after the commencement of mating. Sixteen crosses were made with C strain clones, using washed cultures and timed matings. From replicate mating mixtures of each cross, a sample of 30 pairs was isolated 12 hours after the beginning of mating and another sample of 30 pairs after 36 hours. The results clearly showed a difference in the viability (% immature progeny) of the two series of isolates (Table 3). Moreover, the frequency of mature cultures was 50.8% in the 12 hour-isolates and only 12.9% in the 36 hour-isolates. This difference in frequency suggested that the mature cultures were, in fact, those of Round 1 exconjugants, that they were capable of remating, and that about half of the remated pairs were viable. When the viabilities of the 12 hour and 36 hour pairs were compared, we calculated that 41.7% of the viable pairs at 36 hours were produced by genomic exclusion and 58.3% by normal conjugation.

The logical next step was to select semi-amicronucleate clones from some of the above crosses, to outcross them to a normal clone of a different strain, and to select the mature exconjugants, remate them, and to conduct genetic tests on the progeny. This protocol was similar to one used in testing the AB × C* cross (ALLEN 1967a, b). Two semi-amicronucleate clones were selected: A3-7642c and C-15646a. Each was crossed to a normal clone from the E strain (E-12635), pairs were isolated within 12 hours after mating began, and the two exconjugants from each pair were separated when the pair came apart. Strains A3 and C are H^E/H^E at the *H* serotype locus, while strain E is H^D/H^D.

The viabilities of the crosses were very low—9.4% for A3 × E and 3.3% for C × E. When serotyped, the immature cultures were Hde, and both exconjugants from these pairs were alike in phenotype; thus, these progeny arose as a result of normal conjugation. Out of 138 A3 × E pairs, 24 gave rise to two mature cultures; while out of 150 C × E pairs, 112 sets of cultures were mature. From the two crosses 30 sets of these mature clones were selected. When serotyped, one clone from each set was He in phenotype (like the A3 or C strain parent), and the other clone was Hd in phenotype (like strain E). Thus, each set either represented parental clones or Round 1 exconjugant clones. The two kinds of mature clones would be distinguished by their breeding behavior. When remated, parental clones should give rise to a distribution of progeny similar to the original crosses; that is, mainly mature and dead. Any immature progeny would be Hde. Round 1 exconjugant clones, on the other hand, would have a micronucleus derived from the E strain parent (and H^D/H^D in genotype). When remated, and provided the cross were viable, they should give rise to immature progeny which are Hd.

The 30 sets of clones were remated, 10 from the A3 × E cross and 20 from the C × E cross (Table 4). From each cross 30 pairs were isolated, and the two exconjugants from the same pair were permitted to grow up together in the same depression. Four out of 10 of the crosses of remated clones derived from the A3 × E cross and 14 out of 20 of the crosses of remated clones derived from the C × E cross were inviable, giving rise to either dead or to mature cultures, which were Hde in phenotype. These inviable crosses could have resulted either from

TABLE 4

Genomic exclusion in progeny of inbred mature exconjugants derived from crosses of a semi-amicronucleate and a normal clone

Derivation of exconjugants	No. crosses	No. mature	No. dead	No. immature	Total No. pairs isolated*	Serotypes of: Mature Hde	Serotypes of: Immature Hd
A3 × E	4	15	87	0	102	15	0
	6	26	52	99	177	26	99
C × E	14	19	401	0	420	19	0
	6	14	86	76	176	14	76

* Pairs isolated 12 hours after mating. A3 and C, the semi-amicronucleate parents, are underlined. They are H^E/H^E in genotype. Strain E is H^D/H^D.

the remating of parental cells or from crosses of Round 1 exconjugants which gave rise to lethal Round 2 progeny. The 12 viable crosses gave rise to mature cultures, which were Hde, and to immature cultures, all of which were Hd in phenotype. The appearance of immature progeny which were Hd in these crosses is strong support for the assignment of a minimum of 12 of the 30 sets of mature clones to the category of "Round 1 exconjugants".

The appearance of Round 1 exconjugants and genomic exclusion in outcrosses of normal and semi-amicronucleate clones, as well as the data on inbred strains, suggests that the same basic nuclear mechanism observed in the AB × C* cross also applies here. These data thus give substance to the hypothesis that there is a syndrome of nuclear behavior associated with genomic exclusion and that this syndrome is of general occurrence within syngen 1.

Heritability of the defective micronucleus: Additional evidence for an association between the micronuclear condition of the parents and the frequency of mature progeny came from further observations on Round 1 exconjugants derived from the AB × C* cross. Moreover, after a year of culture, we found that the stability of the micronucleus varied in different exconjugants and that the degree of stability seemed to depend upon the source of the *old macronucleus.*

The breeding performance of 16 sets of Round 1 exconjugants was compared in 1964 (at the time of their genesis) and in 1965 (after approximately 200 fissions). At the same time stained preparations were made of the micronuclei of samples of cells from representative clones. In 1964 the average viability of the 16 crosses was 94% (Table 5). By 1965 it dropped to 56.7%. Most of the decrease in viability was due to a tenfold increase over the course of the year in the frequency of mature progeny—from 3.8 to 37.9%.

In 1964 counts were made of the number and types of micronuclei in samples of approximately 50 to 100 cells of each of the 32 exconjugants. All cells in all samples had diploid micronuclei and 99.6% had only one diploid micronucleus.

In 1965 similar counts were made on larger samples of six of the 16 sets of Round 1 exconjugants and of six sets of *their* mature progeny (Table 6). For comparison, the micronuclear distribution was also determined in samples of AB and C*. After a year of culture, it was apparent that the micronucleus was being

TABLE 5

*Breeding performance of Round-1 exconjugants from AB × C**

Cross	Fall 1964			Fall 1965		
	No. mature	No. dead	Total No. pairs isolated	No. mature	No. dead	Total No. pairs isolated
1a × b	0	4	60	8[A,B†]	1	15
2a × b	1	5	60	13[C,D]	0	15
3a × b	0	0	59	11[E,F]	2	15
4a × b	7	0	60	8	0	15
5a × b	4	0	60	11	0	15
6a × b	0	0	30	10	0	15
7a × b	1	4	49	1	0	15
8a × b	1	1	55	1	1	15
9a × b	0	0	59	2	0	15
10a × b	4	0	59	4	2	15
11a × b	5	1	60	2	0	15
12a × b	7	1	60	7	3	15
13a × b	0	0	60	0	0	15
14a × b	1	2	30	8	3	15
15a × b	0	0	30	5	1	15
16a × b	0	0	30	0	0	15
Total	31	18	821	91	13	240
Percent	3.8	2.2	..	37.9	5.4	..

† Origin of mature exconjugants examined in Table 6.

lost from one of the two Round 1 exconjugant clones of each set. In each set it was the exconjugant clone with the C* phenotype (and, hence, with an old macronucleus derived from C*) that had a deficient micronucleus. In these samples an average of 5.6% of the cells had more than one diploid micronucleus, 3.8% had hypodiploid micronuclei (which were smaller in size than diploid micronuclei) and 7.8% were amicronucleate. In contrast, exconjugant clones with the AB macronucleus (1b, 2b, 3b, etc.) had normal diploid micronuclei. Less than 0.5% had more than one diploid micronucleus, only one cell out of 3000 had a hypodiploid micronucleus, and none were amicronucleate.

Counts were also made of six sets of mature progeny (Aa, b; Ba, b; Ca, b; etc.) that arose in crosses of 1a × b, 2a × b, 3a × b. In these samples all cells had diploid micronuclei (Table 6). Exconjugants with the AB phenotype had only one diploid micronucleus, while 0.7% of the cells with the C* phenotype had two diploid micronuclei.

The micronuclear condition of the Round 1 exconjugants appeared to be less normal in 1965 than in 1964. In the later samples one exconjugant clone of each set contained some cells without a micronucleus or with a defective micronucleus. The viability of their crosses was also lower and a tenfold increase in mature progeny occurred. These data strongly suggest a correlation between the presence of a defective micronucleus in one of the parents and the appearance of mature progeny. They also show that Round 1 of genomic exclusion can occur more than once in the same cells.

TABLE 6

Distribution of micronuclear types in AB, C, Round 1 exconjugants, and mature exconjugants derived from Round 1 exconjugants (Fall 1965)*

Sample†	No. diploid nuclei			No. hypodiploid‡ nuclei			Amicronucleate
	1	2	3+	1	2	3+	
AB	100.0	0	0	0	0	0	0
C*	0	0	0	72.6	10.4	4.6	12.4
Round 1:							
1a*	90.6	3.6	0.6	0	0	0	5.2
b	99.8	0.2	0	0	0	0	0
2a*	61.8	5.2	2.4	14.0	0.8	0.2	15.6
b	99.8	0.2	0	0	0	0	0
3a*	81.8	5.6	1.6	2.2	0	0	8.8
b	98.8	1.0	0	0.2	0	0	0
4a	100.0	0	0	0	0	0	0
b*	85.4	3.4	2.0	3.4	0	0	6.2
5a	99.8	0.2	0	0	0	0	0
b*	91.2	3.4	1.0	0.4	0	0	4.0
6a*	86.0	4.0	1.0	2.0	0	0	7.0
b	99.6	0.4	0	0	0	0	0
Mature exconjugants:							
Aa*§	99.8	0.2	0	0	0	0	0
b	100.0	0	0	0	0	0	0
Ba*	99.4	0.6	0	0	0	0	0
b	100.0	0	0	0	0	0	0
Ca	100.0	0	0	0	0	0	0
b*	97.6	2.4	0	0	0	0	0
Da*	99.8	0.2	0	0	0	0	0
b	100.0	0	0	0	0	0	0
Ea	100.0	0	0	0	0	0	0
b*	99.6	0.4	0	0	0	0	0
Fa*	99.8	0.2	0	0	0	0	0
b	100.0	0	0	0	0	0	0

* Phenotype like C*.
† 500 cells examined in each sample.
‡ Classified as hypodiploid on the basis of size.
§ See Table 5 for origin.

The fact that it was the exconjugant with the C* phenotype that was losing its micronucleus is significant. It implies that the macronucleus either directly or indirectly controls the fate of the micronucleus. Even in newly generated heterocaryons (e.g. mature exconjugants A, B, C, etc.) there may be a tendency for the micronuclear divisions to be less well oriented in relation to cytokinesis in the presence of a C* rather than an AB macronucleus. These data suggest, therefore, that the micronucleus is perpetuated normally in the presence of certain macronuclear genotypes, such as AB, but not normally in the presence of other macronuclear genotypes, such as C*. Introduction of a normal micronucleus by Round 1 of genomic exclusion into a cell previously defective in its micro-

nucleus results in only temporary rescue of the micronucleate condition, unless Round 2 occurs. If Round 2 does not occur, the introduced micronucleus apparently also becomes defective in the presence of the C* macronucleus.

DISCUSSION

The nuclear basis for genomic exclusion in the AB × C* cross appears to be generally applicable to other crosses of syngen 1 clones in which genomic exclusion is observed. A syndrome of associated nuclear behavior was postulated as a necessary prerequisite for the occurrence of genomic exclusion. The first requirement was that one of the parents should have a defective micronucleus. Secondly, in crosses to normal clones, a high frequency of mature progeny should be observed in pairs isolated within 12 hours after the commencement of mating. Thirdly, such pairs, when reisolated, should give rise to immature progeny with only the genes of the normal parent. Portions, or all, of this syndrome were observed in crosses within certain inbred strains of syngen 1, in outcrosses, and in crosses of mature progeny derived from the AB × C* cross.

NANNEY's observations (1963) bear on the genomic exclusion syndrome and its generality in syngen 1. He observed irregularities of genetic transmission in over half of 48 different outcrosses. Most of these outcrosses involved clones of particular inbred strains, ones which were poorly viable, when inbred. Of much interest in the present context is NANNEY's statement (1963) that: "crosses with low viability (few immature progeny) and high frequencies of nonconjugation (mature progeny) are much more likely also to manifest irregular genetic transmission".

One of the poorly viable inbred strains that NANNEY (1963) found provoked genomic exclusion was the B1 strain. A cytogenetic study showed that many clones of this strain had defective micronuclei (NANNEY and NAGEL 1964). Unfortunately, it proved impossible to obtain further genetic data from this strain, so that some of the inferences of this study for the nuclear basis of genomic exclusion proved premature. However, the important point, here, is the fact that most B1 clones were semi-amicronucleate and that genomic exclusion had previously been recorded for outcrosses of this strain.

Is genomic exclusion confined to syngen 1, or does it occur more widely in other syngens of *T. pyriformis*? Unfortunately, very little information on this question is obtainable from the published accounts of breeding experiments in syngens other than syngen 1. The presence of anlagen in stained preparations is often used as a criterion for successful conjugation. Such a criterion does not, however, distinguish between Round 1 of genomic exclusion and true conjugation. Crosses which give rise to immature progeny are a safer index for successful conjugation.

Some syngens (1, 2, 3, 6, 8) do have an immature period (NANNEY and CAUGHEY 1953; HURST 1958; BYRD 1959; GRUCHY 1955; ORIAS 1963). Other syngens (4, 7, 9, 10, 11, 12) do not (GRUCHY 1955; OUTKA 1961; ELLIOTT and KENNEDY 1962; ELLIOTT *et al.* 1962; ELLIOTT, personal communication). In four

of these (syngens 7, 9, 11, 12) the mating types of the two exconjugants are reported to be the same as the parental mating types (OUTKA 1961; ELLIOTT and KENNEDY 1962; ELLIOTT, personal communication). It is entirely possible that genomic exclusion could have occurred in the crosses reported for these syngens, since some of the parental clones clearly had defective micronuclei (e.g., in syngen 9; RAY 1955), and the viabilities of the crosses were low: 12-53% in syngen 7 (OUTKA 1961), 15.9% in syngen 9 (ELLIOTT and KENNEDY 1962), 3 to 4% in syngen 11 and less than 2% in syngen 12 (ELLIOTT *et al.* 1964).

The results of occasional crosses in other syngens could also be interpreted on the basis of genomic exclusion. For example, a case reported by WELLS (1961) involving a tetraploid and amicronucleate clone in syngen 6 could be so interpreted. In the one viable and completely tested pair in which all four first-fission products were recovered, all four caryonides had micronuclei. Two caryonides had the mating type of the amicronucleate partent, the other two, the mating type of the tetraploid parent. The degree of ploidy of the micronuclei in the exconjugants was not mentioned, but they all may have acquired the tetraploid micronucleus, since the mean generation time (of division) of all four caryonides was similar to that of the tetraploid parent.

A prerequisite for genomic exclusion is the presence of a defective micronucleus in one of the parents. Are clones with defective micronuclei common among collections of the wild strains of different syngens? They are, and the most common type of clone collected is amicronucleate. An average of 65% of the clones from the Pacific collections (containing syngens 9, 11, 12) were amicronucleate, 17% were semi-amicronucleate, and 18% were micronucleate (ELLIOTT *et al.* 1964). Amicronucleate clones made up 60% of the Central and South American collections (with syngens 2 and 9; ELLIOTT and HAYES 1955), while in the collections taken from the United States and Canada (containing syngens 1 to 8), 33% were amicronucleate clones (GRUCHY 1955). Finally, 39% of the clones were amicronucleate in collections from Europe (with syngens 3, 4, 6, and 10; ELLIOTT *et al.* 1962). Since amicronucleate clones were collected in locations where each of the 12 syngens have been found, it is clear that the amicronucleate condition is widespread in *T. pyriformis*. However, it is not clear what fraction of the total amicronucleate population is represented because of the techniques involved in collecting the samples (ELLIOTT, personal communication). A sample is taken, powdered milk is added to support the growth of bacteria which serve as a food source for the Tetrahymena, and then clones are initiated. Such a technique obviously selects for those cells which are capable of dividing.

In the laboratory most clones having a defective micronucleus can be propagated by mass transfers of cells; however, cloning often results in early termination of many of these lines. In syngen 1, lines which fail to propagate are often amicronucleate. Many of these, when examined cytologically, appear to be missing an oral apparatus. They are often round or abnormal in shape, which may give rise to their appearance as "crinkled" when viewed under low magnification with the dissecting microscope. In contrast, amicronucleate clones obtained from field collections are often normal morphologically. They propagate well in the

laboratory, usually with fission rates similar to micronucleate clones (ELLIOTT, personal communication).

WELLS (1961) reviewed this problem more extensively. She came to the conclusion that certain micronuclear functions were indispensable to the organism; thus, the amicronucleate condition was usually lethal. However, in viable amicronucleate cells she postulated that these functions were taken over by the macronucleus. Other speculations are, of course, possible, and we wonder if the lethality of newly arising amicronucleate cells in the laboratory is not due simply to the loss of the mouth. Prevention of this loss in certain macronuclear genotypes may lead to the establishment of viable amicronucleate clones.

Observations on heterocaryons (exconjugants with a new diploid micronucleus and an old macronucleus of a different genotype) indicated that the *macronuclear* genotype influenced the behavior of the micronucleus during division. After a year of culture, departures from the normal micronuclear condition were observed more frequently in exconjugants with an old macronucleus derived from a cell previously defective in its micronucleus. These observations suggested that normal micronuclear behavior during division is inherited and that it is under the control of the macronucleus. NANNEY (1957) also found evidence for genetic factors affecting the maintenance of normal micronuclear function in studies of semi-amicronucleate clones. In crosses within and between sets of sister caryonides he found that the frequency of the semi-amicronucleate condition varied and that it was usually higher in the inbred crosses. The pattern of inheritance was complex and suggested that several genes influenced the behavior of the micronucleus. Macronuclear genes were also inferred as influencing micronuclear behavior during conjugation of strain d59 in *Paramecium aurelia* (SONNEBORN 1954).

Loss of the micronucleus can occur at any fission. In normal genotypes the probability of its loss is so low that this event is not observed much before 1000 fissions (NANNEY 1959); however, in other genotypes the probability is much higher so that loss is observed considerably earlier. It is probably the chief form of senility in this organism and leads to death of the cell line either during fission or during conjugation. However, from the frequency of amicronucleate clones in field collections—and the number that appear to be normal—it is clear that loss of the micronucleus can occur without damage to the organism and that compensatory pathways have been developed during the course of evolution to avoid this damage. Under optimal growth conditions amicronucleate clones appear to be equal to micronucleate clones in their vegetative viability. Whether they are equally viable under suboptimal conditions does not appear to have been determined (ELLIOTT, personal communication). Amicronucleate cells are, of course, sexually dead, since they can no longer contribute genes to the gene pool. Thus, unless there are conditions under which they are at a selective disadvantage, the species would be destined to become asexual.

Cells which engage in genomic exclusion have defective micronuclei and also do not contribute genes; however, they do reacquire a micronucleus in which the genes from the normal parent have recombined. Is genomic exclusion an evolu-

tionary accident or does it have survival value for the species? It does permit a "sexually dead" cell to reenter the breeding population, but only by genomic replacement. It does permit recombination of the genes contributed from the normal cell, but only at some hazard to the normal cell, since some of the matings to defective cells do not survive. However, it is possible that the disadvantages may be outweighted by the fact that as a result of genomic exclusion the defective micronucleus is destroyed. Genomic exclusion could be of potential importance to the species since it rids the population of defective cells, and, in so doing, it prevents the development of new viable amicronucleate lines. According to this view, genomic exclusion may be an alternative pathway which was evolved for the reacquisition of a normal micronucleus.

SUMMARY

Genomic exclusion is probably of general occurrence in *Tetrahymena pyriformis*. In syngen 1, it may occur whenever a cell, which is defective in its micronucleus, is mated to a cell with a normal diploid micronucleus. Evidence is reported that there is a high correlation between the presence of a semi-amicronucleate parent in the cross, mature progeny, and the occurrence of genomic exclusion in crosses in syngen 1. These are aspects of a syndrome of associated nuclear phenomena basic to the genetic consequences of genomic exclusion and previously worked out on a cross of AB × C*.—Genomic exclusion has, so far, been unequivocally demonstrated in syngen 1 only, although the results of some crosses recorded in the literature of other syngens suggest that it may occur elsewhere.—Loss of the micronucleus appears to occur often in this organism, since the frequency of amicronucleate clones in world-wide collections is very high. In syngen 1 this loss is influenced by the macronuclear genotype. Since amicronucleate clones are sexually dead, it is proposed that genomic exclusion represents an evolutionary alternative in which the defective micronucleus is destroyed and replaced by a normal micronucleus in which genic recombination has occurred.

LITERATURE CITED

ALLEN, S. L., 1960 Inherited variations in the esterases of Tetrahymena. Genetics **45**: 1051–1070. —— 1963 Genomic exclusion in Tetrahymena: Genetic basis. J. Protozool. **10**: 413–420. —— 1965 Genetic control of enzymes in Tetrahymena. Brookhaven Symp. Biol. **18**: 27–54. —— 1967a Genomic exclusion: A rapid means for inducing homozygous diploid lines in *Tetrahymena pyriformis*, syngen 1. Science **155**: 575–577. —— 1967b Cytogenetics of genomic exclusion in Tetrahymena. Genetics **55**: 797–822.

ALLEN, S. L., S. L. KOCH, and C. A. PATRICK, 1967 Defective micronuclei and genomic exclusion in selected C* subclones. (In preparation).

BYRD, J. R., 1959 The breeding system of variety 3, *Tetrahymena pyriformis*. Dissertation, University of Michigan, Ann Arbor.

DRYL, S., 1959 Antigenic transformation in *Paramecium aurelia* after homologous antiserum treatment during autogamy and conjugation. (Abstr.) J. Protozool. **6** (Suppl.): 25.

ELLIOTT, A. M., and R. E. HAYES, 1953 Mating types in Tetrahymena. Biol. Bull. **105**: 269–

284. —— 1955 Tetrahymena from Mexico, Panama, and Colombia with special reference to sexuality. J. Protozool. **2**: 75–80.

ELLIOTT, A. M., and J. R. KENNEDY, 1962 The morphology and breeding system of variety 9, *Tetrahymena pyriformis*. Trans. Am. Microscop. Soc. **81**: 300–308.

ELLIOTT, A. M., M. A. ADDISON, and S. E. CAREY, 1962 Distribution of *Tetrahymena pyriformis* in Europe. J. Protozool. **9**: 135–141.

ELLIOTT, A. M., M. A. STUDIER, and J. A. WORK, 1964 *Tetrahymena pyriformis* from several Pacific Islands and Australia. J. Protozool. **11**: 370–378.

GRUCHY, D. F., 1955 The breeding system and distribution of *Tetrahymena pyriformis*. J. Protozool. **2**: 178–185.

HURST, D. D., 1958 The breeding system of variety 2, *Tetrahymena pyriformis*. Dissertation, University of Michigan, Ann Arbor.

MELANDER, Y., and K. G. WINGSTRAND, 1953 Gomori's hematoxylin as a chromosome stain. Stain Technol. **28**: 217–223.

NANNEY, D. L., 1957 Inbreeding degeneration in Tetrahymena. Genetics **42**: 137–147. —— 1959 Vegetative mutants and clonal senility in Tetrahymena. J. Protozol. **6**: 171–177. —— 1963 Irregular genetic transmission in Tetrahymena crosses. Genetics **48**: 737–744.

NANNEY, D. L., and P. A. CAUGHEY, 1953 Mating type determination in *Tetrahymena pyriformis*. Proc. Natl. Acad. Sci. U.S. **39**: 1057–1063.

NANNEY, D. L., and J. M. DUBERT, 1960 The genetics of the H serotype system in variety 1 of *Tetrahymena pyriformis*. Genetics **45**: 1335–1349.

NANNEY, D. L., and M. J. NAGEL, 1964 Nuclear misbehavior in an aberrant inbred Tetrahymena. J. Protozool. **11**: 465–473.

NANNEY, D. L., P. A. CAUGHEY, and A. TEFANKJIAN, 1955 The genetic control of mating type potentialities in *Tetrahymena pyriformis*. Genetics **40**: 668–680.

ORIAS, E., 1963 Mating type determination in variety 8, *Tetrahymena pyriformis*. Genetics **48**: 1509–1518.

OUTKA, D. E., 1961 Conditions for mating and inheritance of mating type in variety seven of *Tetrahymena pyriformis*. J. Protozool. **8**: 179–184.

RAY, C., JR., 1955 Irregularities during meiosis in variety nine of *Tetrahymena pyriformis*. Biol. Bull. **109**: 367.

SONNEBORN, T. M., 1954 Patterns of nucleocytoplasmic integration in Paramecium. Proc. 9th Intern. Congr. Genet. Caryologia **6** (Suppl. 1): 307–325.

WELLS, C., 1961 Evidence for micronuclear function during vegetative growth and reproduction of the Ciliate *Tetrahymena pyriformis*. J. Protozool. **8**: 284–290.