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Calcium chloride effects on nuclear development in *Tetrahymena*

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SUMMARY

Conjugating pairs of syngen 1 exposed to 0·1 M-$CaCl_2$ during the time of macronuclear development often abort the new macronuclei and retain the old macronucleus and its associated phenotypes. The new micronucleus is retained, however, so that macro–micro heterocaryons can be constructed. Because the macronucleus is from a sexually mature strain, the heterocaryon is capable of mating immediately, without passing a period of sexual immaturity. Macronuclear abortion may prove a useful short-cut in genetic analysis.

1. INTRODUCTION

Hallet (1972) reported that exposure of conjugating pairs of *Paramecium primaurelia* (formerly syngen 1, *P. aurelia*, Sonneborn, 1975), to sublethal concentrations of $CaCl_2$ modified the course of mating-type determination. Some 20% of the cultures derived from first fission products of pairs of strain 60 treated during macronuclear differentiation mated among themselves, while caryonides in this strain are usually pure and randomly determined for mating type (Sonneborn, 1937). Because of the pattern of distribution of pure sublines in the presumed caryonides, Hallet proposed that mating-type determination was delayed, but occurred before the fifth fission as a whole-nuclear event. Persistently selfing sublines, which would indicate 'mixed', 'mosaic' or 'unstable' macronuclei were not observed.

In the hope that similarly interesting results might be observed during the very similar caryonidal determination in *Tetrahymena* (Nanney, 1956), a preliminary experiment on syngen 1 was undertaken. The results were similar to those of Hallet, but require a different explanation. Indeed they suggest a different interpretation for Hallet's observation. Of more significance, and a justification for a preliminary report, $CaCl_2$ treatment of *Tetrahymena* conjugants appears to abort the macronuclear anlagen while permitting parental macronuclei and zygotic micronuclei to persist. The effect is similar to the induction of 'macronuclear regeneration' by heat treatment in *Paramecium* (Sonneborn, 1950, 1970), and is related to 'macronuclear retention' associated with genomic exclusion in *Tetrahymena* (Allen, 1967*a*).

The micro-macronuclear heterocaryons generated by macronuclear abortion

are sexually mature and are immediately capable of breeding. F_2 and backcross progeny may be produced without the usual delay of 60 or more cell divisions required for maturation. This short-cut could become a routine procedure in association with other recent methodological advances in *Tetrahymena* genetics (Orias & Bruns, 1975).

2. MATERIALS AND METHODS

Two sublines of strain A (A-1768-2*a* and A-1873-6) and three sublines of strain B (B-1868-3, B-1868-4, and B-1868-7), of species 1, *Tetrahymena pyriformis*, were employed. The initial cross of A-6 (mating type VI) × B-7 (mating type VII) was made at 24 °C, axenically in Dryl's solution following the protocols outlined by Orias & Bruns (1975). The initiation of new pairs was blocked 6 h after mixture (3–4 h after the beginning of pairing), by the addition to the mating mixture of 1 % proteose peptone with streptomycin and penicillin. The control pairs were individually isolated into drops of antibiotic proteose peptone to which was added in some cases dimethyl sulfoxide to a concentration of 1 % and/or frozen–thawed cells of various types. These treatments are irrelevant in the present context, though high concentrations of cell lysates may have adverse effects on conjugating cells when applied at critical times. The control cells were isolated between 5 and 7 h after the blocking of pair formation, when conjugation had probably progressed through meiosis and fertilization in most of cells, but before macronuclear differentiation was complete.

The experimental pairs were isolated into the same media to which was added 1·0 M-$CaCl_2$ in Dryl's solution to a final concentration of 0·1 M. The pairs were isolated after the controls, at about 7 h after the blocking of initiation and 13 h after mixture of strains.

The second cross was between A-2 and B-4, but otherwise was carried out in much the same fashion, except that pairs were isolated into bacterized media as well as into proteose peptone.

Three days after pair isolation, all isolations were scored for viability and viable cultures were inoculated into tubes of bacterized media for maturity tests. When observed for mating ability in mixtures with standard testers, synclones can usually be scored as selfers (mature and mating without mixture), mature (mating with all testers except one of the parental types) or immature (normal). The first two categories are usually considered to represent abortive conjugation, with unilateral death in the second case. Earlier experience with such 'non-conjugants' showed that they are usually incapable of subsequent successful breeding performance; they probably arise because of cytogenetic irregularities during conjugation. In the present instance, however, the frequency of such lines was so high among the survivors that macronuclear abortion was suspected. For this reason sublines derived from the original synclones were tested for mating type and then subjected to a subsequent breeding analysis.

This second breeding analysis was carried out in bacterized medium and matings were made to A-2a or B-3. Again 36 pairs were isolated from each of 8 crosses;

the progeny were examined for viability, selfing, and maturity, and were also tested for serotype (Nanney & Dubert, 1960). Strain A is homozygous for the immobilizing serotype gene, H^A, and strain B for the H^D allele.

3. RESULTS

The control pairs isolated from the first cross of strains A × B consisted of 288 pairs, of which 29 (10%) failed to produce a successful synclone. Of the 36 pairs isolated into 0·1 M-$CaCl_2$ 28 died, leaving only 8 viable lines. Of these 6 gave rise to mature synclones composed solely of parental mating types (VI and VII). Thus 75% of the surviving experimentals were 'non-conjugant' while 26 of 259, or 10% of the surviving controls were in this class. Possibly the treatment with $CaCl_2$ is particularly unpleasant to conjugating cells and preferentially selects the pairs which have aborted conjugation. Yet 8/36 (17%) of the total experimentals isolated were non-conjugant, in comparison with the 10% of the controls. Although the experimental sample is small, the high frequency of non-conjugants at least raises a suspicion that the treatment induces rather than merely selects the non-conjugant class.

Table 1. *Viability and maturity in pairs isolated into proteose peptone* (*PP*) *and bacterized peptone* (*BP*) *media 5–7 h after blocking new pair formation*

Medium	$CaCl_2$ Conc. (M)	Death freq.		Mature freq.	
PP	0·00	1/36	0·03	2/35	0·06
BP	0·00	1/36	0·03	0/35	0·00
PP	0·05	7/72	0·10	4/75	0·06
BP	0·05	7/72	0·10	0/65	0·00
PP	0·10	30/72	0·42	15/42	0·36
BP	0·10	40/72	0·55	6/32	0·19
PP	0·15	52/72	0·72	13/20	0·65
BP	0·15	67/72	0·93	4/5	0·80

This question was pursued in a second experiment (Table 1) in which conjugating pairs were isolated into three different concentrations of $CaCl_2$: 0·05 M, 0·1 M, and 0·15 M. Pairs were isolated into proteose peptone medium and also into bacterized peptone media. Pairs in 0·05 M-$CaCl_2$ show some increase in inviability (10% *v.* 3% in the controls), and inviability rises more with higher concentrations. Pairs are slightly more sensitive in the bacterized medium than in the axenic medium. The inviability in $CaCl_2$ was less than in the previous cross in the one comparison possible (78% *v.* 42%), perhaps because of differences in the time or duration of exposure. A preliminary experiment indicates that cells are much more sensitive early in conjugation. At the lowest concentration of $CaCl_2$ used here, the frequency of 'non-conjugation' among the survivors is not different from that of the control, but the proportion rises at 0·1 and 0·15 M. Even in an absolute sense, the number of non-conjugant pairs is higher in the treated than in the control

series. While $CaCl_2$ *may* be less toxic to non-conjugants than to normal pairs, it certainly increases the number of pairs which fail to complete conjugation. Thus far conditions in which all survivors are non-conjugant have not been defined.

Since the interesting question concerns the nuclear equipment of the non-conjugants, 8 different sublines representing 5 of the 6 mature synclones in cross 1 were subjected to breeding analysis (Table 2). A line of type VII from synclone 6 was crossed to strain B. None of the 36 pairs isolated gave defective or mature synclones. Fifteen of these synclones were serotyped and 8 were immobilized by antisera against both parental alleles (Had); 6 were immobilized only by the antiserum against the Hd serotype. (One of the sublines did not react to either antiserum and is still under study; see Nanney, 1962.) Thus, synclone 6 has retained the macronucleus of strain B (responsible for its maturity and its mating type) but its micronucleus clearly carries information from the A parent and segregates the parental alleles 1:1 in a backcross. Synclone 6 is a micronuclear–macronuclear heterocaryon in which the micronucleus is demonstrably heterozygous and capable of normal transmission behaviour many cell generations earlier than under normal circumstances.

Table 2. *Breeding performance of aborted conjugants in backcrosses to parental strains*

Synclone	Mating Type	Crossed to Strain	Progeny synclones			Serotypes			Total
			Died	Selfed	Mature	Ha	Had	Hd	
6	VII	B	0/36	0/36	0/15	0	8	6	14
7	VII	A	7/36	6/28	0/15	9	6	0	15
20	VII	B	4/36	25/32	0/7	0	7	0	7
	VI	A	3/36	29/33	0/4	4	0	0	4
26	VI	A	1/36	1/35	0/15	6	9	0	15
	VII	B	1/36	1/35	0/15	0	9	6	15
28	VII	B	1/36	0/35	0/15	0	8	7	15
	VI	A	1/36	0/35	0/14	7	7	0	14

Synclone 7 was subjected to a similar analysis except that the backcross was to strain A. The viability was not so good and some selfer synclones were produced, but an approximately normal 1:1 segregation was observed. Synclones 26 and 28 were both analysed by backcrosses in both directions, using different sublines in each case. Again the breeding performance is excellent and the segregation ratios are normal.

The exception in this study is synclone 20. Both sublines produced unusually high frequencies of selfing offspring. One subline gave 7 normal offspring from 36 pairs, and the other subline produced only 4. Moreover, both sublines behave as if they are capable of transmitting only the H^A allele. In the backcross to strain B, all the progeny received an H^A allele to give 7 heterozygotes. In the backcross to strain A the four progeny again received the H^A allele from the parent expected to be heterozygous. Both exconjugants from the synclone may have retained a haploid nucleus from the A parent, which only occasionally managed to diploidize

and perform properly its conjugal manoeuvres. The treatment may thus have affected fertilization as well as macronuclear development. On the other hand, synclone 20 may have arisen as a 'spontaneous' aberration, only coincidentally included in the sample chosen.

4. DISCUSSION

Ciliate heterocaryons in which the micronuclei and macronuclei have different genotypes were first identified by Sonneborn (1940, 1947) and used by him (1954*a*) to investigate the function of the micronucleus in *Paramecium aurelia*. Such heterocaryons arise through the abortion of new macronuclei after fertilization. Macronuclear regeneration can be reliably induced in *Paramecium* by heat-treatment of conjugants and by other means, and has played an important role in analysing some problems (Sonneborn, 1954*b*; Berger, 1973).

Heterocaryons are also produced regularly in species 1 of *Tetrahymena pyriformis* when crosses are made between normal strains and certain defective (so-called * or 'star') strains (Allen, 1967*a*, *b*). The composition of the micronuclei in this case is more limited than in *Paramecium*. Because star strains are incapable of producing functional pronuclei, haploid nuclei from the normal mate remain uncombined in both exconjugants. They fail to give rise to new macronuclei at this time, possibly because a diploidization delay disconnects them from their developmental programme. Thus, when the pair members separate, they retain their original macronuclei but have identical, now homozygous, diploid micronuclei. At least one of the 'exconjugants' is usually a heterocaryon, and both are sexually mature because they retain their mature macronuclei. A subsequent conjugation may be induced almost immediately, and the micronucleus now progresses through its entire program, including fertilization and macronuclear development. The processes of 'genomic exclusion' and 'macronuclear retention' have become important techniques in genetic analysis of *Tetrahymena* (Allen, 1967*b*; Allen & Lee, 1971; Orias & Flacks, 1973; Bruns & Brussard, 1974; Orias & Bruns, 1975).

A shortcoming, as well as an important advantage, of heterocaryons derived by genomic exclusion is that the micronuclei are always homozygous. The chief advantage of induced macronuclear abortion is that it can yield heterozygous micronuclei which are capable of immediate analysis through a second mating, without waiting to traverse the normal period of immaturity associated with new macronuclei. The possibilities of induced macronuclear abortion should have been appreciated earlier, and it can certainly be accomplished in other ways; preliminary tests show that it can be induced by heat treatment as in *Paramecium* but the details for optimal induction need to be worked out. The cytological details of macronuclear abortion, the best times of treatment, and the optimal concentrations of reagents must still be determined.

Hallet's observations on *Paramecium*, which provoked these experiments, were interpreted by her as indicating delayed macronuclear differentiation for mating types. The results with *Tetrahymena* are more easily interpreted because

the large number of mating types permits almost certain identification of persisting macronuclei. The use of micronuclear genetic markers also makes clear the origins of the nuclei. Macronuclear regeneration, combined with delayed macronuclear development, is well known in *Paramecium* (Sonneborn, 1954*b*) and might explain Hallet's results without recourse to an effect on mating type differentiation. A repetition of her studies with genetically marked strains would be instructive.

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