J. Cell Sci. 11, 601–609 (1972)
Printed in Great Britain

THE BASIS OF CELL-TO-CELL TRANSFORMATION IN *PARAMECIUM BURSARIA*

I. TRANSFER OF CYTOPLASMIC MATERIAL

C. A. CULLIS*
School of Biological Sciences, University of East Anglia, Norwich, England

SUMMARY

A mating type transformation, involving cell-to-cell contact (abortive conjugation) in syngen 4 of *Paramecium bursaria* has been investigated. By autoradiography, transfer of material between the conjugants in normal and abortive conjugation has been shown to occur. The transfer of this material, which includes protein, RNA and DNA, during abortive conjugation has been shown to be necessary if the transformation is to occur.

The mating type substances produced by sexually competent cells of different mating types have been characterized with respect to their thermal stability. In all cases examined there appeared to be 3 mating type substances produced which had different temperature stabilities. No differences could be shown between the transformed cells and normal cells of the same mating type in relation to stability of mating type substance.

INTRODUCTION

A transformation process has been described in *Paramecium bursaria*, syngen 4, involving cell-to-cell contact (Bomford, 1967). A stock, which is mating type II and which undergoes abortive conjugation with the other 7 mating types of syngen 4, has been isolated and called IIS (separator).

Abortive conjugation is a conjugation process very much shorter than normal conjugation and during which there is no nuclear exchange. The duration of abortive conjugation is up to 6 h while that of normal conjugation is about 36 h. The only nuclear event observed during abortive conjugation is that the micronucleus becomes swollen but this swelling does not persist. Nuclear reorganization (autogamy) does not occur after abortive conjugation (Bomford, 1967).

Following abortive conjugation, the IIS cells pass on to their mates their peculiar characteristics (mating type II and inability to complete conjugation). These newly transformed stocks can be used to transform other paramecia. The cells must be in contact, and in the same relative position that they take up during normal conjugation, if the mating type transformation is to occur. Transformation has not yet been brought about by random physical contact, or by the use of cell-free extracts (Bomford, 1967; Cullis, 1971).

* Present address: John Innes Institute, Colney Lane, Norwich, England.

Table 1. *Stocks of P. bursaria syngen* 4

Stock	Mating type
RB1	I
RB2	II
RB3	III
RB4	IV
RB5	V
BCB4	VI
PS	VII
PG	VIII
amicronucleate PG	IIS

The purpose of this work is to determine the nature of the 'agent' which causes the transformation and the method by which it is transferred from cell to cell. In this paper experiments are described to determine if there is exchange of cytoplasm between mates during abortive conjugation. In an accompanying paper (Cullis, 1972) experiments are presented which attempt to characterize the active cell fraction in molecular terms.

MATERIALS AND METHODS

Stocks and culture methods. Stocks of *P. bursaria* were donated by Dr R. Bomford. A list of the stocks used is given in Table 1. Only one stock of each of the normal mating types was used. The stocks were grown in test tubes in 0·1 % grass infusion, innoculated with *Aerobacter aerogenes* at pH 6·8 (Sonneborn, 1950). All the cells were kept on a light/dark cycle (12 h/12 h) which was necessary for the cells to be sexually reactive.

Matings were carried out in depression slides by mixing a few hundred reactive cells of 2 different mating types at about 11.00 a.m. There was usually an immediate agglutination reaction and pairs were isolated into exhausted culture fluid (Sonneborn, 1950) about 30 min later. After isolation the approximate time of separation of the conjugants was noted. In all cases, the time of the onset of conjugation was taken to be the time of mixing. The exconjugants were either, (i) put on to subbed slides for autoradiography or, (ii) transferred to fresh bacterized medium, grown into clones and tested for mating type.

Radioactive compounds. All were obtained from the Radiochemical Centre, Amersham. These were L-Leucine-T(G), 250 mCi/mM; Uridine-H^3, 3·5 Ci/mM; and Thymine-2-C^{14}, 60 mCi/mM.

Radioactive labelling of cells

[G-^{3}H]Leucine was added, at 50 μg/ml, to a sexually reactive culture 48 h before use. The excess label was removed by repeated washing in Dryl's salts solution (Dryl, 1959) and the animals left in unlabelled Dryl's, for 24 h before use. RNA was labelled by feeding the cells with *E. coli* B grown in infusion in the presence of 1 μCi/ml [^{3}H]uridine. The DNA was labelled by feeding cells with *E. coli* grown in grass infusion containing 1 μCi/ml [2-^{14}C]thymine.

Autoradiography

The exconjugants were placed singly on subbed slides. Slides were subbed by placing them in 0·5 % gelatin, 0·5 % chrome alum and air dried. The cells were air dried, fixed in absolute alcohol:acetic acid (3:1 v/v) for 30 min and rehydrated. Autoradiography was performed using Ilford nuclear research emulsion K5 (Caro & Van Tubergen 1962). Grain counts were

Table 2. *Percentage of cells producing transformed clones after different pairing times while undergoing abortive conjugation*

Mating types used	% transformation after 3·5 h (no. transformed/total no.)	% transformation after 3 h (no. transformed/total no.)
IIS × III	70 (28/40)	2 (1/50)
IIS × V	100 (40/40)	8 (4/50)
IIS × VIII	95 (57/60)	6 (3/50)

made at 1600 × magnification with a grid in one ocular of the microscope. Background counts were taken over an area of 2600 μm^2. Experiments which employed radioactive leucine or uridine were developed after 14 days. Where radioactive thymine was used, the preparations were developed after 28 days.

The stability of the label was tested by treating a series of slides, after fixation, in one of the following ways: (1) extraction with 5% trichloroacetic acid (TCA) at 4 °C for 30 min; (2) extraction with 5% TCA at 95 °C for 25 min; (3) DNase in sodium acetate buffer, pH 5·0, at 37 °C for 4 h; and (4) RNase in sodium acetate buffer, pH 5·0 at 37 °C for 4 h (Pasternak, 1967).

Preparation of cilia

The method used was similar to that of Cohen & Siegel (1963). Sexually reactive animals were concentrated to 50000/ml and lysed by repeated expulsions through a 23-gauge needle. The lysate was cleared by 2 low-speed centrifugations (50 *g*) leaving the cilia in the supernatant.

Reaction with the cilia

About 100 sexually reactive animals were placed in 0·1 ml of culture fluid and 0·06 ml of the cilia preparation added. A coverslip was put on and 25 animals observed in a Zeiss phase-contrast microscope, magnification × 320. The preparation was scored as reactive if 5 or more of the 25 animals had cilia adhering.

RESULTS

Timing of mating type transformation

Matings were set up between cells of type IIS and cells of any of the other normal mating types. Pairs were isolated and time of separation of the exconjugants noted. The 2 exconjugants were grown up separately into clones and tested for mating type. The results showed that if the pairs had remained together for more than 3·5 h then, in every case, the frequency of the transformation to type II was high. If the pairs were together for 3 h or less very few cells were found to be transformed (see Table 2). This suggests that there is a distinct point at which the transformation occurs.

Exchange of macromolecular material during conjugation

Normal conjugation. Cells of mating types III and IV were labelled with [G-^{3}H]leucine and matings were set up between labelled III and unlabelled IV and unlabelled III and labelled IV. Pairs were isolated from the mating mixture and autoradiographed at a known time after mixing. The results are shown in Table 3. It was found that there was a large amount of exchange during conjugation. The exchange started after the

Table 3. *Time of onset of cytoplasmic exchange during normal conjugation. (Mating, labelled III × unlabelled IV)*

Time, h	% of pairs with transfer of label	No. of grains/ 25 μm^2 in cell to which label was transferred	No. of pairs analysed
2·5	0	0	20
3	10	2·0 ± 0·2	20
3·5	50	3·2 ± 0·4	20
4	90	4·0 ± 0·3	20
4·5	100	5·1 ± 0·4	20
5	100	6·3 ± 0·2	20
10	100	12·9 ± 0·9	20
20	100	21·0 ± 3·0	20

animals had been paired for about 3 h. The longer the animals had been paired, the greater the amount of label transferred. This exchange continued for as long as the pairs remained together (see Fig. 1 and Table 3).

Abortive conjugation. Experiments to determine whether or not cytoplasmic exchange occurred during abortive conjugation were carried out with 3 different radioactive labels.

Transfer of proteins

Animals of mating types IIS, III and VIII were labelled and the following crosses carried out – labelled IIS × unlabelled III, labelled III × unlabelled IIS, labelled IIS × unlabelled VIII, labelled VIII × unlabelled IIS. Pairs were isolated. The time of separation of the exconjugants was noted and the exconjugants were then autoradiographed. Similar results were obtained for all 4 of the above crosses. There was a transfer of labelled material, from the labelled cell to the unlabelled cell. The transfer began about 3 h after mixing (Table 4). The longer the pairs were together the greater was the quantity of labelled material transferred and the greater the number of pairs with label (see IIS × VIII). The label was not removed by any of the extraction procedures mentioned in the Materials and methods suggesting that it is incorporated into macromolecular material, probably proteins.

Transfer of RNA

Cells of mating type IIS were labelled with [^{3}H]uridine and the transfer of label during abortive conjugation followed. It was found that there was a transfer of labelled material which began about 3 h after mixing. The labelled material transferred was removed by RNase, or 5% TCA at 95 °C but not by DNase or 5% TCA at 4 °C. Thus there was a transfer of RNA during abortive conjugation.

Table 4. *The appearance of label in unlabelled conjugants, undergoing abortive conjugation, in which one conjugant was labelled with [G-^{3}H]leucine*

Mating types used	Period between mixing and separation of the pairs, h	No. of pairs in which labelled material was transferred	No. of pairs in which no label was transferred
Labelled IIS × unlabelled III	h = 2	0	6
	3 > h > 2	0	7
	4 > h > 3	13	5
	5 > h > 4	4	5
	6 > h > 5	1	0
Labelled III × unlabelled IIS	h = 2	0	15
	3 > h > 2	0	6
	4 > h > 3	13	5
	5 > h > 4	12	4
	6 > h > 5	1	0
Labelled IIS × unlabelled VIII	h = 2	0	3
	3 > h > 2	5	21
	3·5 > h > 3	11	7
	4 > h > 3·5	46	2
	5 > h > 4	75	3
	6 > h > 5	30	0

Transfer of DNA

Cells of mating type IIS were labelled with [2-^{14}C]thymine and the transfer of label, during abortive conjugation to normal cells, followed. It was found that there was a transfer of label which began about 3 h after mixing. The labelled material which was transferred was removed by DNase, or 5% TCA at 95 °C but not by RNase or 5% TCA at 4 °C. Thus there was a transfer of macromolecular material during abortive conjugation. The transfer began about 3 h after the 2 types were mixed, and the material transferred included DNA, RNA and protein.

Transfer of cytoplasmic material and correlation with transformation

The time of the start of cytoplasmic transfer was the same as the time of appearance of transformed cells. This apparent correlation was tested directly. After matings between IIS, labelled with [G-^{3}H]leucine, and unlabelled type VIII, the exconjugants were transferred to fresh bacterized medium and allowed to divide once. One first fission product was used for autoradiography and the other was grown into a clone and tested for mating type. It was found that transformation occurred again after cells had been paired for at least 3 h. Transformation never occurred without the transfer of label, nor was there transfer of label without the sister cell becoming transformed. The transformation occurred irrespective of the quantity of label transferred. Thus cytoplasmic exchange was necessary for the transformation to take place and the transforming agent was transferred soon after cytoplasmic exchange began.

Table 5. *The effect of temperature on the reaction between detached cilia and whole, sexually competent animals*

Source of cilia	Tester stock	Reaction	Mating type substance being inactivated	Temperature to which cilia were heated, °C					
				37	45	50	55	60	70
II	III	*b–B*	*b*	+	−	−	−	−	−
	IV	*c–C*	*c*	+	+	+	−	−	−
	VI	*a–A*	*a*	+	+	+	+	+	−
IIS	III	*b–B*	*b*	+	−	−	−	−	−
	IV	*c–C*	*c*	+	+	+	−	−	−
	VI	*a–A*	*a*	+	+	+	+	+	−
III		*B–b*	*B*	+	+	−	−	−	−
IV		*C–c*	*C*	+	+	+	+	+	−
VI		*A–a*	*A*	+	+	+	+	+	+

+, positive reaction, agglutination observed; −, negative reaction, no agglutination.

Mating type substances of normal and transformed cells

The properties of the mating type substances in syngen 1 were demonstrated by the reaction between the cilia and whole animals (Cohen & Siegel, 1963). They found that the reaction between the detached cilia and whole animals could be prevented by heating the cilia for different lengths of time at 45 °C. Different groupings of mating types required different heating times of the cilia for inactivation of the reaction. Cohen & Siegel (1963) suggested that this was because 4 mating type substances could be produced by paramecia in syngen 1 and these substances had different temperature stabilities.

Cilia from syngen 4 stocks of mating types IIS, II and III, IV and VI, were prepared from sexually reactive animals. The cilia were heated for 10 min at various temperatures between 30 and 70 °C. The results are shown in Table 5. In the reactions between cilia from cells of mating type II it was found that the agglutination with mating type III was absent after heating at 45 °C, with type IV after heating to 55 °C and with type VI after heating to 70 °C. The same pattern of reactivity was observed with the cilia detached from transformed cells. Cilia from mating types III, IV and VI were reacted with types II and IIS; 3 reactions were again observed as defined by heat sensitivity. No differences were obtained in any reaction with either normal or transformed cells.

There are, then, 3 reactions indicated by effects on cilia of different temperatures. The 8 mating types of syngen 4 are generated by pairs of alleles, one dominant and one recessive, at 3 loci, *A*, *B* and *C* (Bomford, 1965). He suggested that the interaction of cells depended upon complementarity between at least one of a pair of substances, for example, *A* on one cell and its complement *a* on another, *B* on one and *b* on the other, or *C* on one and *c* on the other. Cells produce 3 mating type substances, e.g. II produces *a*, *b* and *c* and VII produces *A*, *B* and *C*. The reaction between *B* and *b* is the most sensitive to temperature while that between *A* and *a* is the most stable

(Table 5). Although agglutination reactions between cilia from transformed cells and normal cells occur, it has not been possible to bring about the mating type transformation by the reaction with free cilia (Cullis, 1971; Bomford, 1967).

DISCUSSION

The mating type of syngen 4 stocks of *P. bursaria* is controlled by 2 alleles, one dominant and one recessive at three separate loci called *A*, *B* and *C*. (Bomford, 1965). Each locus is assumed to control the production of a mating type substance which resides on the cilia of sexually competent cells. Normally for a cell to be mating type II, it is homozygous for the recessive allele at all 3 mating type loci. Thus the transformation changes the phenotype in such a way that the dominant alleles are no longer expressed while the recessive alleles do appear to be expressed.

The reactions with the cilia from normal cells of mating type II and cells of type IIS were the same. This suggests that the transformed cells produce the same mating type substances as normal cells of mating type II. The suggestion is further supported by the effect of the transformation on cells which were homozygous for the dominant allele at the *B* mating type locus. In this case, the transformed cells became type II-IIIS, that is they did not mate with type II or with type III (Bomford, 1965, 1967). This can be explained by the absence of any mating type substance whose production is controlled by the *B* mating type locus. The appearance of the type II-IIIS suggests that the transformed cells do produce mating type substances, since if the transformed phenotype was due to a complete lack of mating type substances the II-IIIS phenotype would not be expected. Cells homozygous for the dominant allele at the *C* mating locus transform to type IIS (Bomford, 1965) suggesting that ability to produce the mating type substance controlled by the recessive allele at this locus is introduced into the cell with the transforming agent.

The introduction of this ability is further suggested by the effects of the transformation in syngen 2 of *P. bursaria*. It has been possible to transform certain syngen 2 stocks, due to the intersyngenic reactions between syngens 2 and 4 (Bomford, 1965, 1967). The intersyngenic reactions of transformed syngen 2 stocks, which are different from those of normal syngen 2 stocks (Bomford, 1965, 1967), are compatible with the suggestion that these stocks have acquired the ability to produce the mating type substance controlled by the recessive allele at the *C* mating type locus in syngen 4 stocks.

From the results presented in this paper, macromolecular exchange during abortive conjugation is necessary for the transformation to take place. The transforming 'agent' also enables certain cells to produce a new mating type substance. Experiments designed to determine the molecular nature of the transforming agent are presented in the following paper (Cullis, 1972).

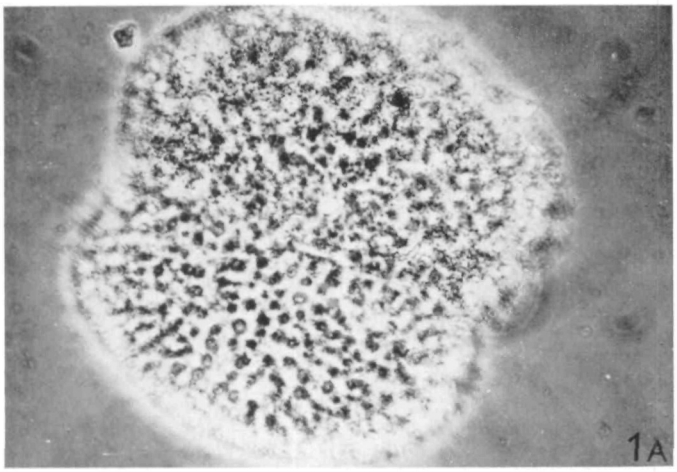
The author gratefully acknowledges the support of the Commonwealth Scholarship Commission, for the award of a Commonwealth Scholarship and the University of East Anglia for a Postgraduate studentship. The help and advice from Dr I. Gibson during this work are also acknowledged.

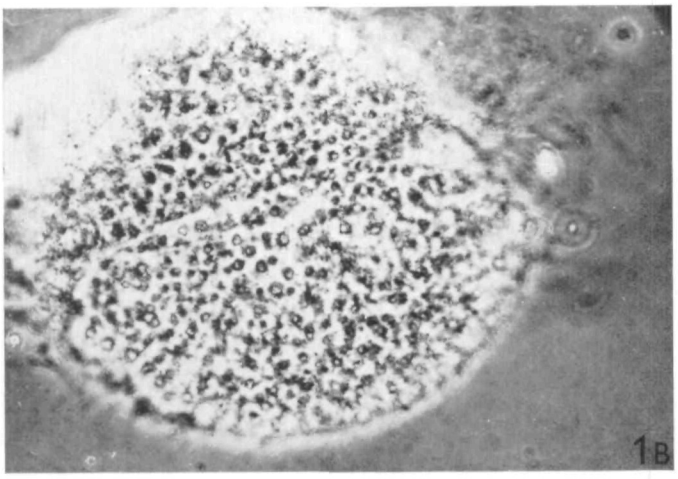
REFERENCES

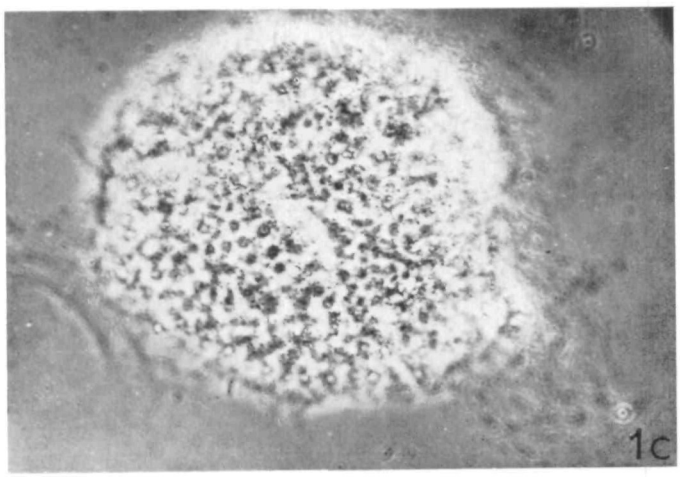
BOMFORD, R. H. (1965). *Genetics of Paramecium bursaria*. Ph.D. Thesis, University of Edinburgh.
BOMFORD, R. H. (1967). Stable changes of mating type after abortive conjugation in *Paramecium bursaria*. *Expl Cell Res.* **47**, 30–41.
CARO, L. G. & VAN TUBERGEN, R. P. (1962). High resolution autoradiography. I. Methods. *J. Cell Biol.* **16**, 173–188.
COHEN, L. W. & SIEGEL, R. W. (1963). The mating type substances of *Paramecium bursaria*. *Genet. Res.* **4**, 143–150.
CULLIS, C. A. (1971). *Transformation in Paramecium bursaria*. Ph.D. Thesis, University of East Anglia.
CULLIS, C. A. (1972). The basis of cell-to-cell transformation in *Paramecium bursaria*. II. Investigation into the molecular nature of the transforming agent. *J. Cell Sci.* **11**, 611–619.
DRYL, S. (1959). Antigenic transformation in *Paramecium aurelia* after homologous antiserum treatment during autogamy and conjugation. *J. Protozool.*, Suppl. **6**, 25.
PASTERNAK, J. (1967). Differential genetic activity in *Paramecium aurelia*. *J. exp. Zool.* **165**, 395–418.
SONNEBORN, T. M. (1950). Methods in the general biology and genetics of *Paramecium aurelia*. *J. exp. Zool.* **113**, 87–143.

(*Received* 23 *December* 1971)

Fig. 1. Cytoplasmic transfer during conjugation between normal cells. Cross involving mating types III and IV. Mating type III was labelled with [G-^{3}H]leucine before the mating was set up. The pair of cells was autoradiographed before the cells parted. A, B, C, 2·5, 3·5 and 6 h after mating set up, respectively. ×960.


1A


1B


1C