ULTRASTRUCTURE OF MEIOSIS-INDUCING
(HETEROTYPIC) AND NON-INDUCING
(HOMOTYPIC) CELL UNIONS IN
CONJUGATION OF BLEPHARISMA

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SUMMARY

Cells of mating types I and II of Blepharisma japonicum interact with each other and unite in heterotypic (type I-type II) or homotypic (type I-type I, type II-type II) pairs. Heterotypic pairs undergo meiosis and other nuclear changes of conjugation, while homotypic pairs remain united for days without the nuclear changes taking place. We compared cell unions of these two kinds of pairs at the ultrastructural level. In the homotypic union, cell membranes are closely juxtaposed, separated by a distance of about 20 nm. This arrangement is interrupted in some places by vacuoles and small cytoplasmic bridges. Saccule-like structures tend to be more abundant near the united surfaces. Microtubules running at right or slightly obtuse angles with the cell surface (PACM microtubules) are characteristically present at the united region of cells. These structures are very similar to those observed in earlier stages of the heterotypic union. However, in homotypic pairs, cells unite only at the anterior half of the peristome, while in heterotypic pairs cells unite also at the posterior half of the peristome, where the cell membrane totally disappears in later stages. PACM microtubules persist for at least 18 h in homotypic unions, while they disappear within a few hours in heterotypic unions. These differences between the two kinds of cell union are discussed in relation to the initiation mechanism of meiosis and other nuclear changes of conjugation. Similarities between homotypic union and cell junctions in multicellular organisms are also discussed.

INTRODUCTION

Cell union during conjugation of Blepharisma japonicum is induced by the interaction between complementary mating types I and II. Type I cells secrete gamone I (blepharmone), a glycoprotein (Miyake & Beyer, 1974; Braun & Miyake, 1975), which specifically transforms type II cells so that they can unite. Type II cells secrete gamone II (blepharismone, calcium-3-(2'-formylamino-5'-hydroxybenzoyl) lactate (Kubota et al. 1973)) which similarly transforms type I cells.

Transformed cells can unite in pairs in all 3 possible combinations of mating types, but only heterotypic pairs (type I-type II) complete conjugation. Homotypic pairs (type I-type I, type II-type II) may be united for a day or even longer, but the nuclear

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This characteristic feature of homotypic pairs provides a unique opportunity to investigate the initiation mechanism of meiosis and other nuclear changes in conjugation by contrasting the two kinds of pairs (Miyake, 1975; Miyake, Maffei & Nobili, 1977). It also provides an opportunity to investigate the mechanism of cell union without complications due to the further progress of conjugation (Miyake & Honda, 1976). To exploit these possibilities, we compared the ultrastructure of homotypic and heterotypic unions. So far only the heterotypic union has been described at the ultrastructural level (Ototake, 1969; Miyake, 1970). Part of this work has been reported in abstract form (Bedini, Lanfranchi, Nobili & Miyake, 1974).

**MATERIALS AND METHODS**

**Cells**

Wild-type clones 3β1 (mating type I), D3S (mating type II) of Bangalore strain and clone A1 (mating type I) of the albino strain (Chunosoff, Isquith & Hirshfield, 1965) of *B. japonicum v. intermedium* (Hirshfield, Isquith & DiLorenzo, 1973), formerly *B. intermedium* (Bhandary, 1962), were used. Clones 3β1, D3S and A1 are designated R1 (red, mating type I), Rn (red, mating type II) and A1 (albino, mating type I) respectively. Cells were grown in lettuce medium inoculated with *Aerobacter aerogertes*, concentrated, washed with and suspended in SMB, a salt solution for *Blepharisma* (Miyake & Honda, 1976) and used after 1–2 days. Cultures were maintained and all experimental procedures with living cells were performed at 24 ± 1 °C.

**Gamone**

Gamone 2 was synthesized blepharismone (Tokoroyama, Hori & Kubota, 1973) purified as indicated by Kubota et al. (1973). The stock solution was a gamone 2 solution in SMB with 1–6 × 10⁴ U./ml activity. The unit activity is defined as the smallest amount of gamone activity that can induce at least one homotypic cell union in 500–1000 cells suspended in 1 ml SMB (Miyake & Beyer, 1973).

**Induction of cell unions**

Homotypic cell union of R1 and A1 was induced by mixing a cell suspension with the stock solution of gamone 2 in a 99:1 ratio. Heterotypic cell union was induced by mixing the suspensions of A1 and Rn. Pairs produced by ciliary union were isolated as they were formed and fixed after selected incubation times.

**Electron microscopy**

Cells were fixed at 24 ± 1 °C in a 1:2:5 mixture of 50 % glutaraldehyde, 4 % osmium tetroxide and 0.13 M sodium phosphate buffer, pH 7.4, dehydrated with an ethanol series and embedded in an Epon-Araldite mixture. The transverse sections were stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop I A electron microscope. At least 3 pairs were observed for each fixation.
RESULTS

Cell surface participation in cell union

When A₁ and R₁ were mixed or A² and R² were treated with gamone 2, cells started uniting by their cilia at the anterior part of the oral side of the cell (ciliary union). This occurred after a waiting period of 1-1-5 h, after which characteristic association between cells is detectable. The ciliary union lasts 1-1-5 h, after which cells unite more intimately by a direct contact of cell bodies (glued union). These results generally conform to previous observations (Miyake & Beyer, 1973; Miyake & Honda, 1976), but the degeneration of cilia, which was briefly mentioned by Miyake & Beyer (1973), was not confirmed in this work.

![Diagram of the peristome](image)

Fig. 1. Diagrammatic illustration of the peristome, which consists of AZM (1), UM (2), ant-UM cilia (3) and peristomal floor (4). The peristome is arbitrarily divided into 3 zones I, II and III. For abbreviations see text.

The region of cells participating in these unions is the peristome. The peristome of this ciliate consists of the adoral zone of membranelles (AZM), the undulating membrane (UM), a row of cilia anterior to UM (anti-UM cilia) and a stripe of non-ciliated cell surface (peristomal floor) which is surrounded by them (Fig. 1). Both AZM and UM are differentiated ciliary structures of the mouth region. The ciliary union is formed by a specific contact between the AZM of one cell and the ant-UM cilia of the other (Honda & Miyake, 1976). Pairs united by ciliary union invariably separated when fixatives were added. The glued union is formed by a direct contact of the peristomal floors of 2 cells. In this work only the glued union was investigated.

In both heterotypic and homotypic unions, the glued union is first formed at the anterior region of the peristomal floor in the proximity of ant-UM cilia (zone I in Fig. 1). The union then extends towards the AZM and posteriorly. The heterotypic
union nearly reaches the posterior end of the peristome, while the homotypic union rarely goes beyond the anterior half of the peristome (zones I and II in Fig. 1).

**Homotypic cell union**

Homotypic pairs were fixed 1, 3, 8 and 18 h after the formation of the ciliary union. Initially the glued union may extend over only a few μm (Fig. 2). Cell membranes at the united area are closely juxtaposed, separated by a distance of about 20 nm. The membrane is about 8 nm thick and internally lined at a separation of about 12 nm by another membrane which is discontinuous in many places. Below this lining there are, as in other parts of the cortex, pigment granules, mitochondria, bundles of microtubules running parallel to the longitudinal axis of the cell (cortical microtubules) and saccule-like structures. The latter appear to be more abundant at the region of cell union. In addition, microtubules running at right or slightly obtuse angles to the cell surface are present (see also Figs. 3–6). These microtubules, PACM (perpendicularly associated with the cell membrane) microtubules (Miyake, 1978), are found only at the united region and in close proximity to it. They are not detectable in cells which have not encountered cells or gamones of the complementary type.

In 3-h-old pairs, the united surfaces are more extensive and more indented (Figs. 3, 4). The juxtaposition of the membranes is interrupted by vacuolar spaces which are mostly surrounded by the cell membrane alone, without the inner lining described above. Cytoplasmic bridges 0.1–0.2 μm in width are formed, often adjacent to the vacuolar spaces (Fig. 5). PACM microtubules are more conspicuously visible. They appear to be directly attached to the inner lining of the cell membrane and form tufts (Fig. 5).

In 8- and 18-h-old pairs, the indentation of the united surfaces is more conspicuous (Fig. 6) and cytoplasmic bridges are broader, reaching 3 μm in width (Fig. 7). Inside the vacuolar spaces and at the outside of the cell near the united region, there are many filaments and/or granules. PACM microtubules are still present.

In respect to these changes, no difference is detectable between R1-R1 and A1-A1 pairs, except that the changes are slightly less extensive in the latter.

Pairs usually separate 1–2 days after the beginning of gamone treatment, but on rare occasions the united area broadens and cells then join permanently. At the united region of such fused pairs, cell membranes and PACM microtubules are no longer visible but granules and filaments are still present at the outside of the cell, near the united region (Fig. 8). Meiosis and other nuclear changes of conjugation were never observed in these pairs.

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Fig. 2. Cross-section of 1-h-old, R1-R1 homotypic pair at zone I. The narrow contact area involves the juxtaposition of the membranes closely adjacent to the ant-UM cilia (aum) on the peristomal floors. Pigment granules (pg), cortical microtubules (cmt) and saccule-like structures (ss) are present. × 48,000.

Fig. 3. Cross-section of 3-h-old, A1-A1 homotypic pair at zone II. The glued membrane area is larger and appears more indented. Vacuolar spaces (v) are formed along the juxtaposition line. PACM microtubules (arrows) are clearly visible, together with pigment granules (pg), mitochondria (m), saccule-like structures (ss) and ant-UM cilia (aum). × 20,000.
Fig. 4. Cross-section of 3-h-old, R1–R1 homotypic pair at zone II showing the same features as in Fig. 3. The same abbreviations are used. × 12000.

Fig. 5. Cross-section of 3-h-old, R1–R1 homotypic pair at higher magnification. The vacuolar spaces (v) between the juxtaposed membranes are mostly surrounded only by the plasma membrane. A narrow cytoplasmic bridge (small arrow) and tufts of PACM microtubules (large arrows) are visible. × 50000.
Heterotypic cell union

Heterotypic pairs were fixed 3, 6, 8, 13, 16, 18, and 24 h after formation of the ciliary union. In 3-h-old pairs, the united area already extends the whole length of the peristomal floor. The cell membranes at the contact area are separated by a distance of 20 nm (Fig. 9). The cell membrane, the inner lining of the cell membrane, pigment granules, mitochondria, PACM microtubules and saccule-like structures at the united region are very similar to those observed in homotypic unions. Limited membrane breakdown occurs all through the united region, producing cytoplasmic bridges 0.1–0.2 μm in width between the partners. By and large these cytoplasmic bridges are more numerous and slightly wider than in homotypic pairs. PACM microtubules are abundant, but some of them appear partly depolymerized, with fuzzy contours and sinuous shape (Fig. 10). In some places aggregations of fibrous material, which is probably the degradation product of PACM microtubules, are seen.

In the pairs older than 3 h, PACM microtubules are no longer visible, while the aggregation of fibrous material persists in 6- and 8-h-old pairs. In pairs older than 16 h, a large cytoplasmic bridge up to 30 μm wide is formed at the posterior part of the peristomal floor (Fig. 11). In one of three 24-h-old pairs examined, the membrane had disappeared along the whole length of the united region.

DISCUSSION

Homotypic and heterotypic unions have been considered similar in the following respects: (1) both unions are induced by gamone (Miyake, 1968); (2) they look alike under the optical microscope (Miyake & Beyer, 1973); (3) in both of them cells start uniting by ciliary adhesion (Miyake & Beyer, 1973) between the AZM of one cell and the ant-UM cilia of the other (Honda & Miyake, 1976); (4) their resistance to pronase increases in the same way (Miyake & Beyer, 1973). Our observations at the ultrastructural level also reveal many similarities between the two kinds of unions. However, there are two clearly detectable differences: (1) PACM microtubules disappear within a few hours in the heterotypic union, while in the homotypic union they persist much longer; and (2) the cytoplasmic bridge between cells is formed more extensively in the heterotypic union. Since meiosis and other nuclear changes of conjugation occur only in the heterotypic pairs, these morphological differences might provide clues to the initiation mechanism of these nuclear changes.

PACM microtubules have not been described in non-conjugating cells of Blepharisma (cf. Jenkins, 1973). Our own observation on non-conjugating and non-gamone-treated cells also failed to detect such structures. Therefore, these microtubules must be newly formed in conjugating cells. Ototake (1969) briefly reported the presence of such microtubules at the posterior part of the heterotypic union of B. japonicum (then B. intermedium) fixed within 6 h after the formation of the pair. Our observations indicate that they start degenerating 3 h after the formation of the ciliary union and completely disappear after a further 3 h. On the other hand, they persist much longer in the homotypic union. Thus degeneration of these microtubules might be correlated
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Fig. 8. Cross-section of 1-day-old, A1–A1 homotypic pair at zone II. The membranes of the glued union have completely disappeared, resulting in a large cytoplasmic bridge. PACM microtubules are no longer visible. Numerous filaments and granules outside the cells are present at the fusion area. × 4000.

Fig. 9. Cross-section of 3-h-old, A1–R heterotypic pair at zone III. The glued union is similar to that of homotypic pair. A cytoplasmic bridge (cb), some PACM microtubules (arrows) and many saccule-like structures (ss) are visible, besides pigment granules (pg) and mitochondria (m). × 16000.

Fig. 6. Cross-section of 8-h-old, A1–A1 homotypic pair at zone II. In this section the glued union shows very deep indentations (di) and a large vacuolar space (v). PACM microtubules (arrows), pigment granules (pg), saccule-like structures (ss), and mitochondria (m) are visible. Outside the united area there are numerous filaments and granules. × 20000.

Fig. 7. Cross-section of 18-h-old, A1–A1 homotypic pair at zone II. A fairly large cytoplasmic bridge filled with mitochondria (m) is visible in the centre. The vacuolar space on the right contains numerous filaments. PACM microtubules (arrow) are still clearly visible in association with the juxtaposed membranes. × 32000.
Fig. 10. Cross-section of 3-h-old, A¹-R³ heterotypic pair at zone I. A high magnification to show the depolymerization of PACM microtubules (arrows). Many saccule-like structures (ss) are present. × 60000.

Fig. 11. Cross-section of 18-h-old A¹-R³ heterotypic pair at zone III. The united area consists of a large cytoplasmic bridge in which saccule-like structures (ss), pigment granules (pg) and other material (arrow) are present. × 4000.
with initiation of meiosis and other nuclear changes of conjugation. PACM microtubules have not been reported in conjugation of other ciliates, but this may be due simply to the fact that the early stages of conjugation have rarely been investigated at the ultrastructural level.

The extent of cell union and the extent of membrane breakdown provide by far the largest differences between the two kinds of union. However, with regard to the initiation mechanism of the nuclear changes, the large cytoplasmic bridge at the posterior part of the peristome in the heterotypic union should be excluded from consideration, because of its late occurrence; the heterotypically united cells are irreversibly determined to undergo meiosis and other nuclear changes (activation) within 1–2 h after the beginning of ciliary union (Miyake et al. 1977). What remains are the slightly larger number and size of the cytoplasmic bridges in the heterotypic union at the early stage of the pair formation. Whether such a relatively minor difference is a determining factor for the occurrence or non-occurrence of activation needs further investigation.

At this point it should be noted that the contact area of the heterotypic union extends to nearly the whole length of the peristome, while that of the homotypic union usually stops halfway. This suggests that the glued union at the posterior part of the peristome might provide specific information for the activation.

Our observations on the heterotypic union of *B. japonicum* generally confirm those reported in earlier works and also solve a discrepancy between them. Ototake (1969) noticed an extensive breakdown of the cell membrane all through the united region in a pair fixed 12 ± 3 h after pair formation, while Miyake (1970) reported that the extensive membrane breakdown is limited to the posterior part of it. We found that both phenomena may occur. But the breakdown of the membrane along the whole length of the peristome appears to be a rare event, since it was observed in only one out of 15 of 12–24-h-old pairs.

The close juxtaposition of cell membranes at the united region, with narrow cytoplasmic bridges (up to a few μm wide) is similar to that observed in conjugation of other ciliates, e.g., *Paramecium aurelia* (Jurand & Selman, 1969), *P. caudatum* (Vivier & André, 1961), *P. multimicronucleatum* (Inaba, Imanoto & Suganuma, 1966) and *Tetrahymena pyriformis* (Elliot & Zeig, 1968). The extensive breakdown of the membrane resulting in cytoplasmic bridges wider than 5 μm is also observed in *P. aurelia* (Schneider, 1963), *Euplotes crassus* (Nobili, 1967) and *Oxytricha* sp. (Ricci, Banchetti, Nobili & Esposito, 1975). In these respects the cell union in conjugation of *B. japonicum* conforms to that of other ciliates.

How cell union is induced by gamone is largely left for future study. However, the extensive cortical changes described above suggest that gamone induces cell union by evoking a series of changes in cells which eventually give them the capacity to unite, rather than by serving as a binding material between cells. This is consistent with the conclusion of Miyake & Honda (1976) that gamone induces protein synthesis and protein synthesis is needed for the cell union.

Finally, it may be worthwhile to compare the homotypic cell union of *Blepharisma* to some of the cell junctions in multicellular organisms, particularly the desmosome
junction. There are similarities between them such as the regular juxtaposition of cell membranes, separated by about 20 nm, the presence of tufts of filamentous structures emanating from the united surfaces, and the persistence of the union for hours or days without total fusion of cells. The reversibility of the union may also be comparable. The significance of these resemblances will be made clear by investigating both types of cell union at the molecular level, since it is at this level that the uniformity of life is most explicitly expressed. Homotypic cell union, which can be regularly induced by a single substance in a homogeneous population of cells, is amenable to such analysis.

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REFERENCES


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