Conjugation of *Paramecium tetraurelia* cells: selective wheat germ agglutinin binding, reversible local trichocyst detachment and secretory function repair*

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* This paper is dedicated to Professor Hellmuth Sitte on the occasion of his 60th birthday

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Summary

Conjugation of ciliated protozoa involves precisely defined stages. In *Paramecium* there occur: (1) the formation of ciliary contacts; (2) local loss of cilia and formation of narrow contacts between anterioventral somatic cell membranes; followed by (3) formation of small cytoplasmic bridges (which allow for the exchange of molecular components only); before (4) their enlargement enables the cells to exchange micronuclei. This work concentrates on stages (2) and (3) in *P. tetraurelia* cells. We analysed lectin binding during conjugation, using mainly secretory mutants *nd6* and *tl* (occasionally *nd9-28°C* and *7S* (wild-type) cells); (*tl* is a trichocyst-free mutant, *nd* strains are non-discharge mutations). Our most essential findings are: already early contact sites (stage (2)) can be labelled with wheat germ agglutinin–fluorescein isothiocyanate; only early stages (2) can be reversed with an excess of WGA; no selective labelling was found with concanavalin A–fluorescein isothiocyanate (commonly observed with other ciliate species); WGA binding sites most probably involve GlcNAc residues. As known before, trichocysts disappear from contact sites in stages (2)/(3). We then analysed this process in more detail. Trichocysts are locally detached from the cell membrane (without transfer to the other conjugant) and re-inserted after conjugation. From our data obtained with *nd6/tl* conjugants one can conclude that trichocysts (in particular secretory lectins or glycoproteins contained in them) cannot play a regulatory role in the conjugation process, since conjugation precedes secretory function repair. We also present evidence for a secretory function repair in *nd* mutations by *tl* cells (though *tl* cells are devoid of any recognizable 'exocytotic apparatus'). *nd6* cells are more difficult to 'cure' than *nd9-28°C*. The 'curing effect' seems to emanate from the conjugation zone. This might indicate the occurrence of diffusible 'curing factors' exchanged mainly in stage (3). Finally, we show that secretory function repair in *nd* cells induces the assembly of 'fusion rosettes', i.e. the formation of the wild-type ultrastructure of the trichocyst release sites, to about the same extent as *nd* cells are functionally repaired (visualized by the release of trichocysts in response to two different trigger agents).

Key words: conjugation, lectins, membranes, mating, *Paramecium*, wheat germ agglutinin.

Introduction

When ciliated protozoan cells of opposite mating types are mixed under appropriate conditions, they may undergo conjugation, which eventually permits the exchange of micronuclei. The establishment of cell–cell contacts involves: (1) the formation of ciliary contacts (Takahashi *et al.* 1974; Vivier, 1974; Kitamura & Hiwatashi, 1976); then (2) the loss of cilia, with the formation of somatic contact sites; which permits (3) the occurrence of multiple, small (0·2 μm) membrane fusion sites (for reviews, see Watanabe, 1978; Miyake, 1981; Hiwatashi & Kitamura, 1985). Expansion of the initial fusion sites to an elaborate

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broad cytoplasmic bridge finally permits (4) the exchange of micronuclei. (For micrographs of conjugation stages, see Results.) These structural features are, on the whole, similar in *Paramecium* (Schneider, 1963; Inaba *et al.* 1966; Vivier, 1974; Watanabe, 1978) and in *Tetrahymena* (Wolfe, 1982, 1985).

The conjugation site on the somatic cell membrane of most ciliates analysed to date selectively binds concanavalin A (ConA) (*Tetrahymena*: Ofer *et al.* 1976; Frisch & Loyter, 1977; Suganuma *et al.* 1984; Wolfe *et al.* 1984; Pagliaro & Wolfe, 1987; *Euplotes*: Lueken & Oelgemöller, 1985). However, we show here for the first time the implication of wheat germ agglutinin (WGA) binding sites in *Paramecium tetraurelia* cells. No defined diffusible mating inductors (gamones) that could account for WGA staining are known for *Paramecium* cells (cf. Miyake, 1981; Hiwatashi & Kitamura, 1985; see also Concetti *et al.* 1986).

Besides the characterization of conjugation sites with regard to their lectin-binding properties, we have tried to determine if there is any involvement of secretory organelles (trichocysts) in the conjugation process, as has previously been claimed (see Discussion).

Another aspect we investigated was the functional repair of *nd* mutations ('non-discharge', unable to release trichocysts due to a defect in membrane fusion capacity) by another mutant strain (*tl*, 'trichless'), which is devoid of trichocysts. This enables us to exclude any possible organelle transfer more strictly than in previous experiments, for which wild-type cells had been used. *nd9-28°C/tl* pairs also allowed us to analyse by freeze-fracturing the assembly of ultrastructural components during secretory function repair.

Materials and methods

Cell material

Paramecium tetraurelia cells of wild-type (*7S*, stock 51s), non-discharge mutant strains *nd6* (Cohen & Beisson, 1980; Lefort-Tran *et al.* 1981) and *nd9* grown at 28°C (*nd9-28°C*; Beisson *et al.* 1976), or *tl* (Pollack, 1974; 'trichless', with no trichocysts) were used. They were grown at 25°C, exept for *nd9*, grown at 28°C. 'Non-discharge mutants' contain trichocysts docked in the cell cortex, which, however, they cannot release by exocytosis (Beisson *et al.* 1976). The medium was an extract from dried lettuce with $5\,\mu g\,ml^{-1}$ stigmasterol added and inoculated with *Enterobacter aerogenes* (Sonneborn, 1970). The cell cultures grew to a density of 3000–5000 cells ml^{-1} and were used for conjugation experiments when they had just reached early stationary phase. The cells were left in their culture medium and equal parts (with the same number of cells) of the different mating types were mixed in order to induce conjugation. This occurs spontaneously, since the strains used obviously belong to different complementation groups (see also Lefort-Tran *et al.* 1981); chemical induction was not used. By light-microscopic inspection of conjugants we ascertained that they were always *nd/tl* or *7S/tl* pairs. Conjugation experiments were carried out at room temperature (≈21°C). Usually, about 50–70% of the cells participated in clumping, while only about 10% underwent fusion, about 2 h after mixing of the cells. 10^{-4} M-Ca^{2+} was present throughout these experiments.

Chemicals

The following chemicals were used: cacodylic acid, sodium salt·$3H_2O$ (Serva, Heidelberg, FRG), ConA (E·Y Laboratories, San Mateo, USA), ConA–fluorescein isothiocyanate (FITC) (Medac, Hamburg, FRG), glutaraldehyde (GA) (Serva, Heidelberg, FRG), *N*-acetylglucosamine (GlcNAc) (Sigma, St Louis, MO, USA), malic acid (Merck-Schuckhardt, Hohenbrunn, FRG), *N*-acetylneuraminic acid (NeuAc) from *Escherichia coli* (Sigma), neuraminidase from *Clostridium perfringens* (Boehringer-Mannheim, FRG), picric acid (Merck, Darmstadt, FRG), paraformaldehyde (Polaron Equipment Ltd, Watford, GB), Tris (Paesel, Frankfurt, FRG), WGA (E·Y Laboratories), WGA–FITC (Medac).

Fixation

Stock solutions containing 2% GA and 5% formaldehyde (FA) in 0·1 M-cacodylate·HCl buffer (pH 7·0) were used. (For controls living cells were also analysed.) When fixation was carried out in 2·5% FA, one part of the cells was mixed with one part of 5% FA. For combined fixation in 2·5% FA and 0·5% GA (final concentrations) two parts of FA were mixed with one part of GA and one part of the cells. Fixation was always carried out at room temperature.

Fluorescence labelling

We used FITC conjugates of ConA or WGA as markers, both contained in 0·01 M-phosphate-buffered saline (PBS) (pH 7·45).

ConA–FITC labelling: living conjugating or non-conjugating cells were incubated in ConA–FITC ($0{\cdot}2\,mg\,ml^{-1}$) + 1 mM-$MnCl_2$ + 1 mM-$CaCl_2$ for 2·5 h and washed with 5 mM-Tris·HCl buffer (pH 7·0).

WGA–FITC labelling: living cells were incubated in WGA–FITC ($0{\cdot}1\,mg\,ml^{-1}$) for 10 min and washed in 10 mM-Tris–maleate buffer (pH 7·0).

Other cells were fixed with 2·5% FA for 3 min, washed in 10 mM-Tris–maleate buffer (pH 7·0) with 50 mM-glycine added, incubated in $0{\cdot}1\,mg\,ml^{-1}$ WGA–FITC for 10 min and washed again as described above.

Specificity tests for WGA–FITC labelling

WGA treatment. Living cells were preincubated with $1\,mg\,ml^{-1}$ WGA for 3 min, before WGA–FITC was added in a final concentration of $0{\cdot}1\,mg\,ml^{-1}$. After 10 min cells were washed in 10 mM-Tris–maleate buffer (pH 7·0). One group of cells was observed alive, the other was first fixed in 5% FA (3 min) and then observed.

Sugar treatment. Sugars (NeuAc, GlcNAc) were dissolved in 40 mM-Tris·HCl buffer (pH 7·0). Equal parts of

200 mM-GlcNAc and WGA–FITC (1 mg ml^{-1}) were preincubated for 5 min until cells were added (final concentrations: 20 mM-GlcNAc, 0·1 mg ml^{-1} WGA–FITC); 25 min later, cells were washed in 5 mM-Tris·HCl buffer (pH 7·0) and observed in the living state. NeuAc was tolerated by the cells only at a concentration of up to 2 mM (final concentration).

Neuraminidase treatment. Living cells were exposed to the enzymic activity at pH 7·0 (28°C). Neuraminidase was dissolved in 10 mM-Tris–maleate buffer (pH 7·0) at a concentration of 1 mg ml^{-1} (≙ 0·6 units mg^{-1}). Cells were washed in the same buffer and incubated with 0·5 mg ml^{-1} neuraminidase (≙ 0·3 units ml^{-1}) at 28°C for 1·5 h. A sample of the cells was incubated with 0·1 mg ml^{-1} WGA–FITC for 15 min before they were washed in 10 mM-Tris–maleate buffer (pH 7·0) and observed. Another sample of the cells was fixed with 2·5 % FA for 1 min and incubated with WGA–FITC as described above.

Colloidal iron staining. Conjugating *nd6* and *tl* cells were fixed (2·5 % FA + 0·5 % GA) and stained with colloidal iron according to Hale (1946), as specified by Pearse (1968).

Effects of lectins and sugars on the course of conjugation

WGA and ConA. The following experiments were carried out using *nd6/tl* and *7S/tl* conjugants and lectins in concentrations of 0·04 mg ml^{-1} (WGA) or 0·2 mg ml^{-1} (ConA + 1 mM-$MnCl_2$ + 1 mM-$CaCl_2$). Cells contained in culture medium were mixed with one of the lectins: (1) 15 min before the different mating types were mixed, (2) during the mating reaction, or (3) while holdfast union was taking place. Cells were observed for up to several hours.

NeuAc and GlcNAc. Similar experiments were carried out with GlcNAc (67 mM) and NeuAc (5 mM).

Induction of exocytosis

Saturated picric acid was used as indicated by Beisson *et al.* (1976). Alternatively, equal parts of cells and a 0·01 % solution of aminoethyldextran (AED) (Plattner *et al.* 1984, 1985) were mixed, yielding a final concentration of 0·005 % AED; only this method results in complete trichocyst discharge.

Light-microscopic techniques

Equipment and techniques for fluorescence and phase-contrast (Figs 1, 2) microscopy were as outlined by Kersken *et al.* (1986). Alternatively, bright-field or Nomarski differential interference contrast (Figs 3–8) microscopy was used. Most images presented in this paper are oriented with the anterior ends of the cells towards the right.

Freeze-fracture electron microscopy

For these experiments we applied methods previously described (Pape & Plattner, 1985; Pouphile *et al.* 1986) to *nd9-28°C/tl* conjugants, 6 h after mixing. It was important to use glutaraldehyde-fixed (and glycerinated) cells, since this permits visualization of 'rings' and 'rosettes' (particle assemblies on preformed exocytosis sites, typical of the wild-type ultrastructure; cf. Plattner, 1987) on the same (cytoplasmic) fracture face (Lefort-Tran *et al.* 1978). In contrast to unfixed frozen cells (Plattner *et al.* 1973) this allows one to identify potential exocytosis sites more easily. Freeze-fracture replicas obtained by standard methods were evaluated by counting the number of cells displaying rings with a rosette.

Results

Site and group specificity of lectin labelling

As shown in previous reports (Lüthe & Plattner, 1986; Lüthe *et al.* 1986) the cell surface of vegetative *P. tetraurelia* cells is not distinctly labelled by ConA–FITC; labelling by WGA–FITC is more pronounced. Here we show that the fusion zone of conjugating cells is especially strongly labelled by WGA–FITC (Fig. 1) in *nd6/tl* pairs. This holds also for *7S/tl* pairs (data not shown). The fluorescence is inhibitable by WGA as well as by GlcNAc (Fig. 2) but not by adding NeuAc or by neuraminidase treatment (Table 1). Furthermore, no strong acidic groups (e.g. –COOH, as with NeuAc; Sherbet *et al.* 1972) could be detected in the fusion zone by colloidal iron staining (data not shown). Therefore, we conclude that WGA is bound to the conjugation zone by GlcNAc, rather than by NeuAc residues. These specificity tests were necessary because WGA binds not only GlcNAc but also NeuAc (Bhavanandan & Katlic, 1979; Peters *et al.* 1979).

Structural identification of conjugation stages

Typical images of conjugation stages (see Introduction) are shown in Figs 3 (mating reaction = ciliary interaction), 4A (holdfast union = early conjugation), 4B,C (advanced conjugation) and 4D (cell separation). The whole conjugation process requires 8–12 h (Vivier, 1974), whereas our analyses were conducted over time periods (see figure legends) shorter than those required for nuclear exchange.

Effects of WGA on the course of conjugation

In contrast to WGA, ConA had no effect on the course of conjugation (Table 1). The mating reaction (stage (1), ciliary interaction) is inhibited by WGA only to a small extent (if at all) even when WGA is added to the cells before the different mating types were mixed. In contrast, fusion of pairs (stage (2)) is prevented when WGA is added during the mating reaction. These experiments were carried out with *nd6/tl* and *7S/tl* pairs with essentially identical results. Conjugants seem to establish cytoplasmic connections about 2 h after mixing, as inferred from the occurrence of the first functional repair effects (see below). These lectin effects are summarized in Table 1.

Localized trichocyst detachment

As already reported (Schneider, 1963; Vivier & André, 1961), trichocysts are absent from the fusion zone of conjugating *Paramecium* cells. How this occurs had not been elucidated.

Fig. 1. WGA–FITC labelling of *nd6/tl* conjugants, 4 h (A,B) and 7 h (C,D) after mixing cells. Note that WGA–FITC staining of the somatic cell membrane starts on the anterioventral contact site during the holdfast union stage (A,B) and later spreads over the whole contact site, extending through the entire ventral region. *cr*, fluorescent intracellular crystals; *ci*, cilia. Bars, 10 μm.

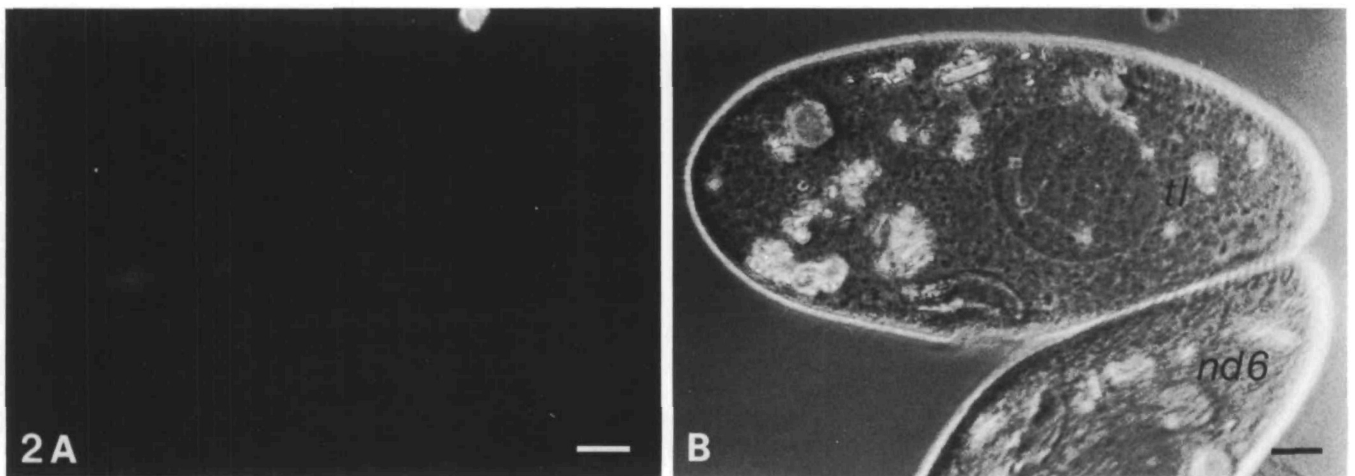

Fig. 2. Control using *nd6/tl* conjugants exposed for 25 min to WGA–FITC saturated with GlcNAc (0·1 mg ml^{-1} WGA–FITC + 0·02 M-GlcNAc). The anterioventral contact site of these conjugants (4 h after mixing) is not stained. Bars, 10 μm.

The following experiments were carried out with *nd6/tl*, *nd9-28°C/tl* and *7S/tl* pairs. Before and during the mating reaction nearly all trichocysts are docked in the cell cortex; few free trichocysts can be detected in the cytoplasm (Fig. 3). No trichocysts can be seen to be extruded from the conjugation zone, even in wild-type cells – again in contrast with earlier suggestions (Vivier & André, 1961). When the cell membranes of the paired cells begin to fuse (≈2 h after mixing the different mating types), docked trichocysts were observed to leave the fusion zone. Fig. 4 shows, for *nd6/tl* pairs, that these trichocysts are detached from the cell membrane and released into the cytoplasm of the *nd6* cell. A similar situation occurs with *7S* cells in *7S/tl* pairs (Fig. 5). At later stages of pairing the fusion zone enlarges and the number of free trichocysts increases (Fig. 4B). When the conjugation zone has reached its maximum extent (≈7 h after mixing the two mating types), the number of free trichocysts is also at its maximum. At this stage, about 150–300 free trichocysts can normally be detected in the trichocyst-bearing cells. A rough calculation shows that this corresponds to the number of trichocysts that is normally docked at this surface area. No trichocysts were normally seen to be transferred from a trichocyst-bearing partner into *tl* conjugant cells (Figs 4C, 5; yet see Fig. 4D). When the cells separate (>8 h after mixing the different mating types) the empty docking sites, contained in the conjugation zone, are occupied again by trichocysts, while the number of free trichocysts decreases until only a few free trichocysts are left in the cells (Fig. 4D). These results are identical for mutants that cannot undergo extrusion (*nd*) and wild-type (*7S*) cells, when they conjugate with a *tl* cell. We conclude that trichocysts are detached from and later on reinserted into the cell cortex.

Functional repair of exocytotic competence

During conjugation of *nd6/tl* and *nd9-28°C/tl* cells, some of the trichocysts of *nd6* and *nd9-28°C* cells become extrudable (when supplied with a trigger agent; see Materials and methods). With *nd6/tl* and *nd9-28°C/tl* pairs, the first trichocysts can be extruded ≈2 h after the beginning of fusion. This occurs only rarely during 'holdfast union' (starting ≈2 h after mixing of the different mating types). Functional repair begins near the fusion zone, first at the anterior end, and later spreading to the posterior end of the cell (Fig. 6B). Such regional differences are particularly evident with *nd6/tl* pairs; they are less evident, though, with *nd9-28°C/tl* pairs (Fig. 7), possibly because functional repair is much faster in this case. About 4 h after the start of cell fusion trichocysts can be extruded from all over the cell, to a smaller extent in *nd6* conjugants (Fig. 6C) and to a much larger extent in *nd9-28°C* cells (Fig. 7C). We mainly used picric acid as a triggering agent, since this permits recognition of extruded trichocysts more clearly, because they do not detach from the cell body. Upon triggering with aminoethyldextran, *nd9-28°C* conjugants can finally extrude all docked trichocysts (Fig. 8B). Again this percentage is much smaller with *nd6* conjugants, although this strain contains almost twice the number of docked trichocysts as *nd9-28°C* cells (Plattner *et al.* 1985). At this time no exchange of genetic material would have occurred (see Discussion).

For controls, non-conjugating cells, which are abundantly present in the conjugation mixture, were also analysed with picric acid (Figs 6A, 7A) or with

Table 1. *Effects of lectins and inhibitory sugars on conjugating cells*

	WGA	GlcNAc	NeuAc	WGA–FITC	ConA	ConA–FITC
Effects of lectins and sugars on the course of conjugation	Does not influence mating reaction but inhibits conjugation (fusion) at 0·04 mg ml^{-1}	None (tested up to 65 mM)	None (tested up to 5 mM)	(see WGA)	No effect on conjugation (0·2 mg ml^{-1} tested)	(see ConA)
Localization of lectin binding sites				Labelling of conjugation zones (0·1 mg ml^{-1})		No labelling (0·2 mg ml^{-1} tested)
Inhibition of WGA–FITC staining				Inhibition by GlcNAc (20 mM), no inhibition after neuraminidase treatment or by adding NeuAc (20 mM)		

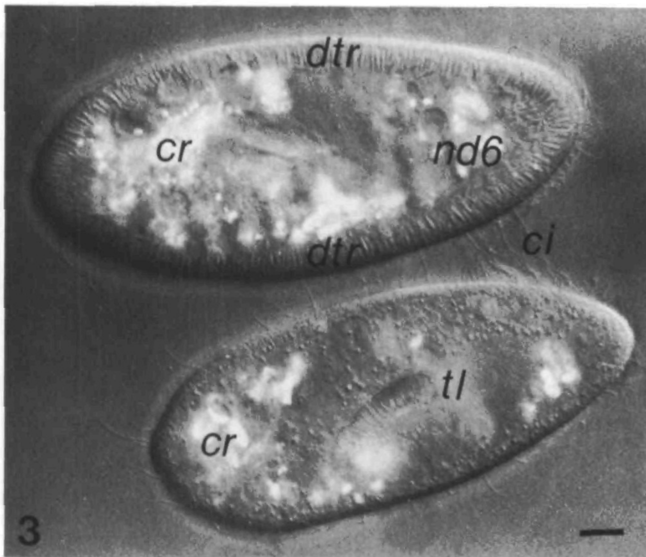

Fig. 3. *nd6* and *tl* cells during mating reaction (stage (1) = ciliary interaction). Note the absence of trichocysts in the *tl* cell, whereas the *nd6* cell contains numerous trichocysts docked onto the cell membrane (*dtr*, visible as 4 μm long rods), but only a few free trichocysts in the cytoplasm (on the right side). *ci*, cilia; *cr*, crystals. Bar, 10 μm.

aminoethyldextran (Fig. 8A) at different time periods after mixing the two mating types. As was to be expected, non-conjugating cells (except for the wild type) were always unable to extrude trichocysts.

Finally, we found by freeze-fracturing that conjugation with *tl* cells causes the assembly of 'fusion rosettes' in *nd9-28°C* (Fig. 9). The resulting freeze-fracture appearance is therefore the same as it would be in normally secreting strains (Beisson *et al.* 1976; Pouphile *et al.* 1986). In controls one finds only 'rings' (*nd9-28°C*) or 'parentheses' (i.e. collapsed rings; *tl*) without rosette particles, as shown before by Beisson *et al.* (1976). Among the *nd9-28°C* cells analysed, ≈10 % displayed this ultrastructural transformation (though this did not include all exocytosis sites in one cell). This percentage is correlated with the number of *nd9-28°C* cells undergoing conjugation and functional repair. The maximal number of particles assembled in a rosette (Fig. 9) was seven, a number never observed in *nd9-28°C* cells from controls (fig. 9 of Beisson *et al.* 1976). A possible sequence of events during rosette assembly is presented in Fig. 9C–F.

Discussion

Selective WGA labelling

One of our essential findings is that, in *P. tetraurelia*, conjugation sites (within the somatic cell membrane) selectively bind WGA (Table 1). This is substantiated by selective WGA–FITC staining and by the capacity of a large excess of WGA to inhibit this staining and to dissociate cells at an early stage (2) of conjugation (holdfast union). The inhibitory effect of an excess of WGA appears possible only when the cell membranes are further apart than the distance required for lectin cross-linking. Concomitantly, cells of late stage (2) (see Introduction and Results) could no longer be affected by WGA. Inhibition of conjugation by WGA has also occasionally been observed by J. Beisson (personal communication). This result corresponds to the observation of conjugation inhibition by ConA in those ciliates whose contact sites stain with ConA–FITC, e.g. *Tetrahymena* (see Ofer *et al.* 1976; Frisch & Loyter, 1977).

Our conclusion, that WGA–FITC staining of conjugation sites is specific for GlcNAc rather than for NeuAc, is supported by biochemical analyses. These revealed the absence of NeuAc residues from the surface membranes of *P. tetraurelia*, at least in vegetative cells (Merkel, 1980).

Our observations on WGA binding are also remarkable insofar as previous studies with related species, such as *Tetrahymena* (Ofer *et al.* 1976; Frisch & Loyter, 1977; Wolfe *et al.* 1984; Suganuma *et al.* 1984; Pagliaro & Wolfe, 1987) and *Euplotes* (Lueken & Oelgemöller, 1985), had demonstrated the involvement of ConA, rather than WGA, binding sites in conjugation. Even the mating zone of the flagellate *Chlamydomonas* is ConA-specific (Millikin & Weiss, 1984). There have been no previous studies using fluorescently labelled lectins with *Paramecium*. On the basis of the inhibitory effects of ConA on conjugation in *P. caudatum*, Tsukii & Hiwatashi (1978) inferred the probable involvement of ConA-binding sites in the conjugation process. This is contrary to our findings using *P. tetraurelia* and for this reason we have carried out extensive specificity tests (see Table 1). (We should like to stress that we did not analyse the effect of ConA on cell agglutination *via* ciliary membranes. As mentioned, WGA exerts no or little effect on this pre-conjugational stage.)

Formation of conjugation sites

Although we have not yet analysed in detail the formation of the WGA–FITC-stainable conjugation sites, we have made a few observations of possible relevance. Despite the large number of cells analysed it is still unclear whether the WGA–FITC-stainable (WGA-inhibitable) 'stripes' found on the anterioventral somatic cell membrane of some *7S* and *nd6* (but not *tl*) cells (data shown for wild-type cells by Lüthe & Plattner (1986)) represent assembly stages of conjugation zones. In the anterior cell region of some *tl* cells we also found WGA–FITC-labelled 'dots', and some *tl* or *nd6* cells displayed WGA–FITC-stainable 'caps' around the anterior apex (data not shown).

Interestingly, the latter patterns were mainly seen after mixing with the other conjugant strain, but before conjugation occurred. More work is required to analyse any possible connection with the reorganization of the cell surface during mating. Preliminary evidence of similar phenomena in *Tetrahymena* has been presented by Feng & Wolfe (1986). For more details, see also the following section.

Functional aspects: could trichocysts or their contents be involved in the regulation of conjugation?

As documented in Figs 4 and 5, trichocysts disappear from the cell contact sites during conjugation. We also present evidence that they are internalized rather than discharged. The formation of *nd/tl* pairs is another argument against any active role for trichocyst components in cell pairing.

However, even quite recent work suggests the direct, local involvement of extrusomes in ciliate conjugation (Suganuma *et al.* 1984; Tiedtke, 1984), both authors using *Tetrahymena,* however. For *Euplotes*, Verni *et al.* (1978, 1981) emphasized the possible importance of the secretion from ampules, which are associated with ciliary roots. In contrast to *Blepharisma* (Miyake & Beyer, 1974) or *Euplotes* (Concetti *et al.* 1986), no defined diffusible mating substances ('gamones', e.g. glycoproteins) are known for *Paramecium* (Vivier, 1974; Miyake, 1981; Hiwatashi & Kitamura, 1985). We do not know where WGA-staining materials of conjugation zones originate in *Paramecium*, how they reach the conjugation sites (see above) and how they are

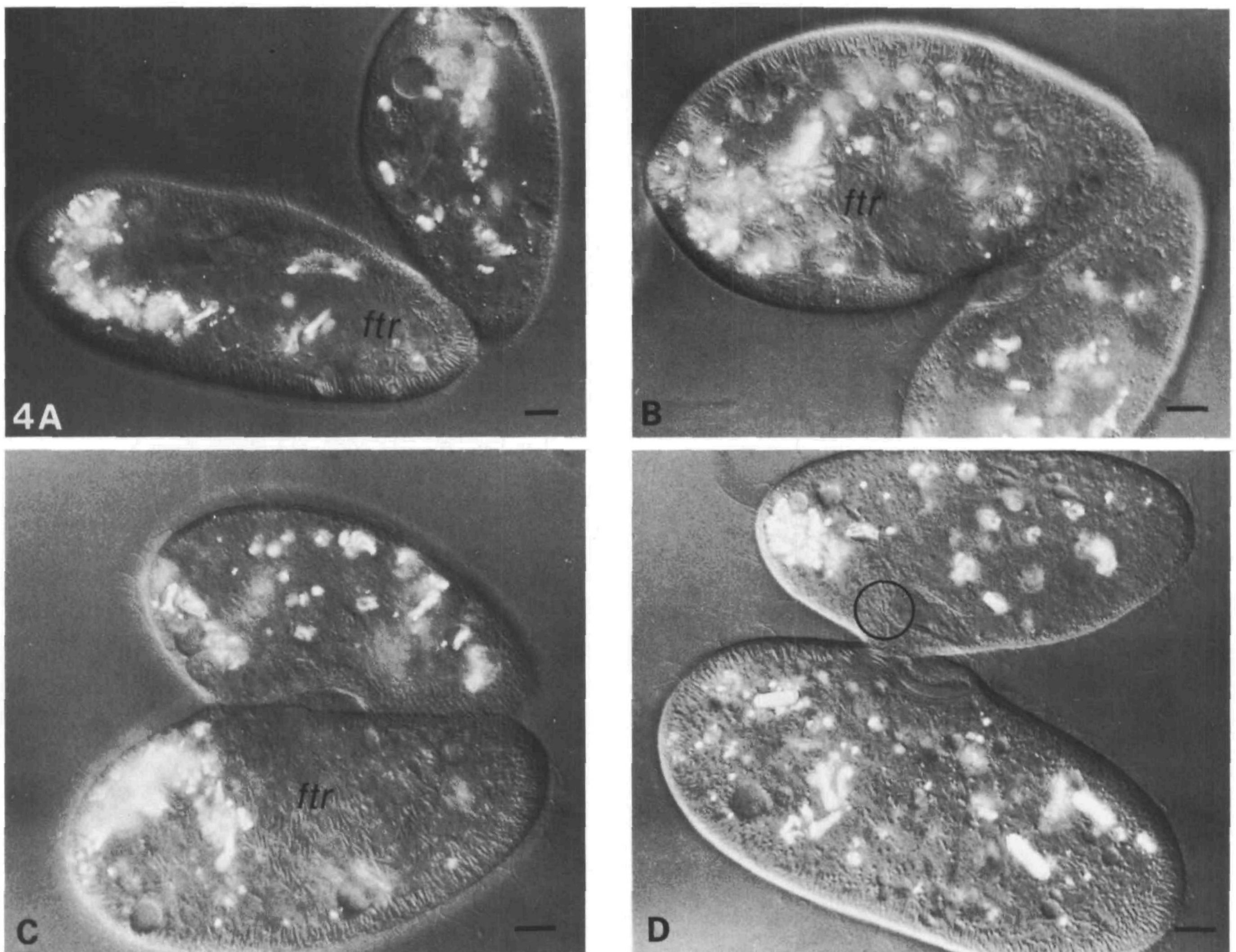

Fig. 4. These *nd6/tl* conjugants, taken 3 h (A), 4 h (B), 7 h (C) and 8 h (D) after mixing the cells, show different stages of conjugation: A, holdfast union; B,C, advanced conjugation; and D, separation of cells. Note the detachment of trichocysts from the cell membrane over the smaller (A), larger (B) or extensive (C) contact site (causing an increasing number of free trichocysts, *ftr*) and the re-docking when conjugants separate (D). Also evident is the absence of trichocyst transfer (A–C). Trichocysts also do not appear in *tl* cells during advanced conjugation (C) and only a few may be seen during late conjugation (D; encircled area). Bars, 10 μm.

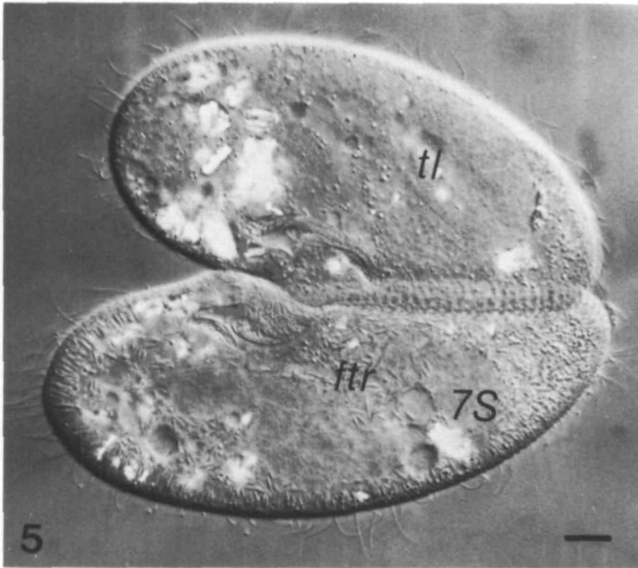

Fig. 5. Conjugating wild-type (*7S*) and *tl* cells (7 h after mixing) show the same reversible local detachment of trichocysts (*ftr*) from the conjugation zone, as shown in Fig. 4 for *nd6/tl* pairs. Bar, 10 μm.

anchored there. Our experiments with *nd/tl* pairs make it unlikely that the secretory lectins newly discovered in trichocysts (Lüthe *et al.* 1986; Haacke-Bell & Plattner, 1987) would be engaged in forming somatic cell contacts during conjugation.

Since WGA *per se* is not glycosylated (Goldstein & Hayes, 1978) this also excludes any possible involvement of an (unknown) surface membrane lectin in the conjugation process. Glycolipids also appear unlikely as WGA binding sites, since conjugation can be stopped at stage (2) by exposing *P. aurelia* cells to trypsin (Yashima, 1985). Glycoprotein components of the cell surface coat (but hardly adsorbed trichocyst components) appear to be the most likely molecular equivalents of conjugation determinants.

Secretory function repair

The limited transfer of molecular components (but not of organelles) from one partner to another during conjugation has been known for a long time (see below). 'Microinjection' *via* conjugation had been applied before by Satir *et al.* (1986) with *Tetrahymena*, and by Cohen & Beisson (1980), Beisson *et al.* (1980) and Lefort-Tran *et al.* (1981) with *Paramecium* cells, with the aim of analysing in different strains the competence of docking and release of trichocysts. (In a parallel approach these authors have transplanted, with similar results, trichocysts (and cytoplasm) by true microinjection, using different donor and recipient strains. This endorses the value of conjugation experiments for functional repair studies.) Strain *nd6* has been determined to be deficient in a cell surface component, whereas a defect in a cytosolic component was assumed for *nd9-28°C* (Aufderheide, 1978; Cohen & Beisson, 1980; Lefort-Tran *et al.* 1981). In contrast, the inability of *tl* cells to assemble trichocysts could not be 'cured' in the early stages of conjugation with wild-type cells (Lefort-Tran *et al.* 1981).

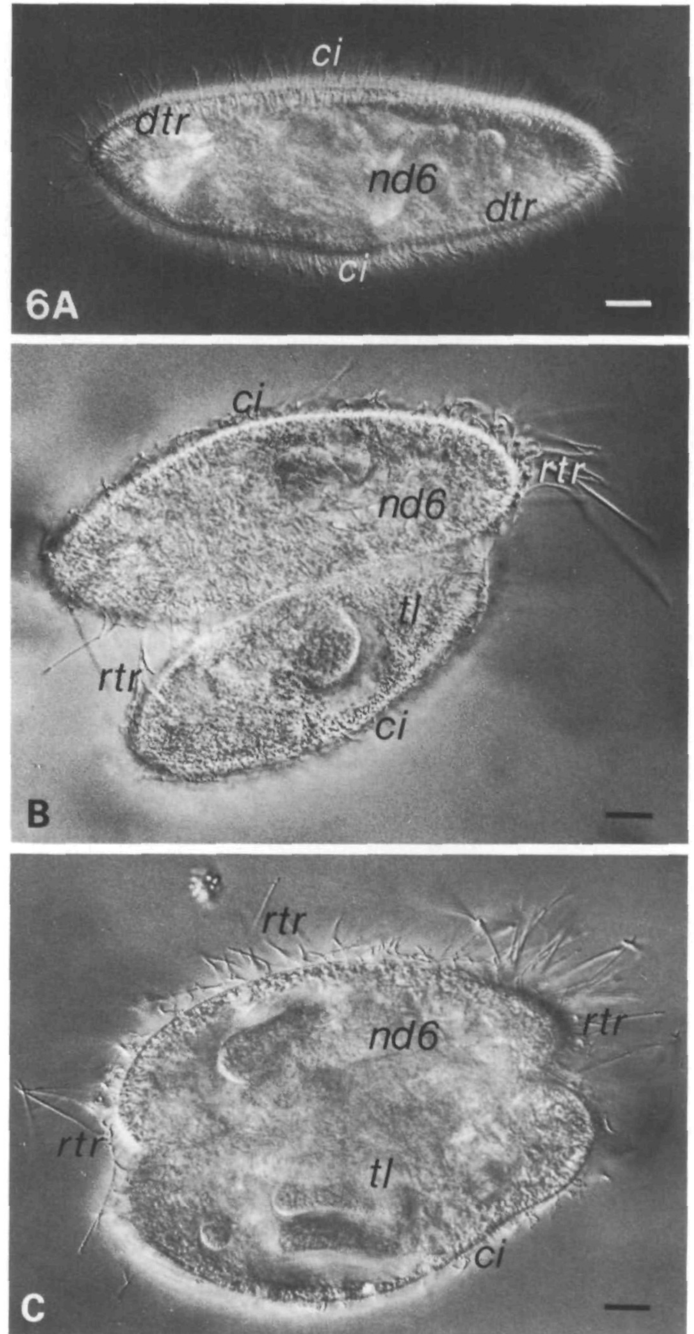

Fig. 6. Functional repair in *nd6* cells by conjugation with *tl* cells. Picric acid was used to stimulate exocytosis of trichocysts. Released trichocysts (*rtr*) remain stuck in the cell after their contents have expanded during exocytosis. A. Control *nd6* cell, unable to release its trichocyst (*dtr*). B,C. At 4 and 6 h after mixing *nd6* and *tl* cells. Note the preferable release of trichocysts from the anterior end (with the longest time period available for cell–cell contact and fusion period). *ci*, cilia. Bars, 10 μm.

repair (see below). Different 'exocytotic fusion-mediating factors' may be required for *nd6* and *nd9-28°C* cells: we found that, in the case of *nd6*, secretory function repair starts at the conjugation site and slowly spreads from there throughout the surface. It appears possible, in agreement with the data from Cohen & Beisson (1980) and Lefort-Tran *et al*. (1981), that with *nd6* cells curing factors would have to diffuse along the cell membrane. The assumption of the requirement of cytosolic curing factors (which could rapidly diffuse) in *nd9-28°C* cells (see Beisson *et al*. 1980) would also be compatible with our data showing a rather rapid functional repair over the whole cell circumference of *nd9-28°C* cells.

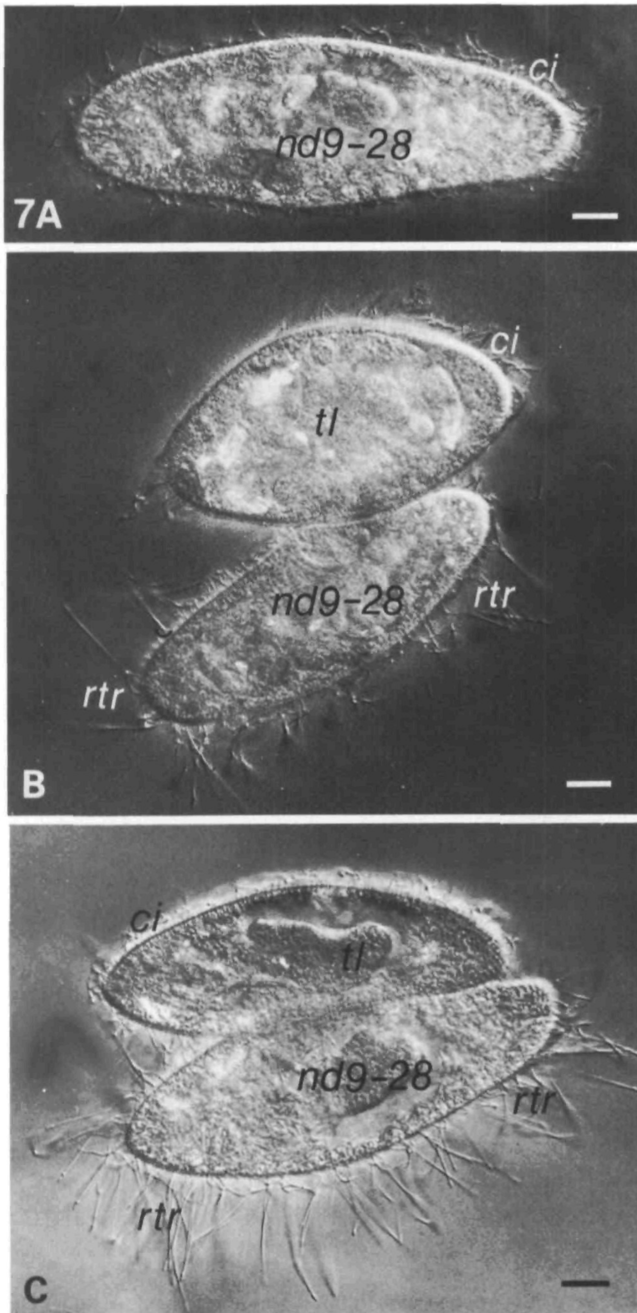

Fig. 7. Same conditions and time periods as in Fig. 6, but using *nd9-28°C/tl* conjugants. A. Showing the inability of single *nd9-28°C* cells to release trichocysts in response to picric acid. B,C. Showing secretory functional repair in *nd9-28°C* cells 4 h (B) or 6 h (C) after mixing with *tl* cells. Again released trichocysts (*rtr*) remain stuck in the cell body. *ci*, cilia. Bars, 10 μm.

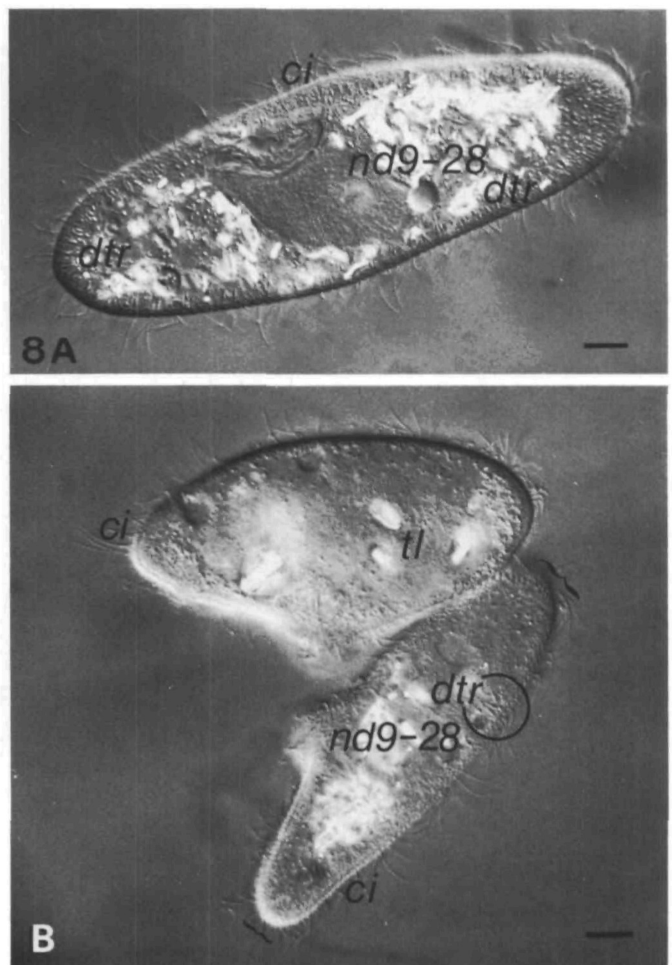

Fig. 8. As in Fig. 9, *nd9-28°C* cells (A is the control) were combined with *tl* cells (B, 3 h after mixing). Secretory function repair was monitored here by adding aminoethyldextran as a secretagogue, which causes the complete release of the majority of trichocysts from the *nd9-28°C* cell (e.g. at sites shown by brackets); only a few trichocysts are left behind (e.g. at *dtr*), whereas secreted trichocysts have separated from the cell and, therefore, are no more visible. *ci*, cilia. Bars, 10 μm.

We can now add new data showing that *tl* cells also contain cytoplasmic components required for the development of the exocytotic membrane fusion potential. The use of *nd/tl* pairs was also a prerequisite for the analysis of ultrastructural events during functional

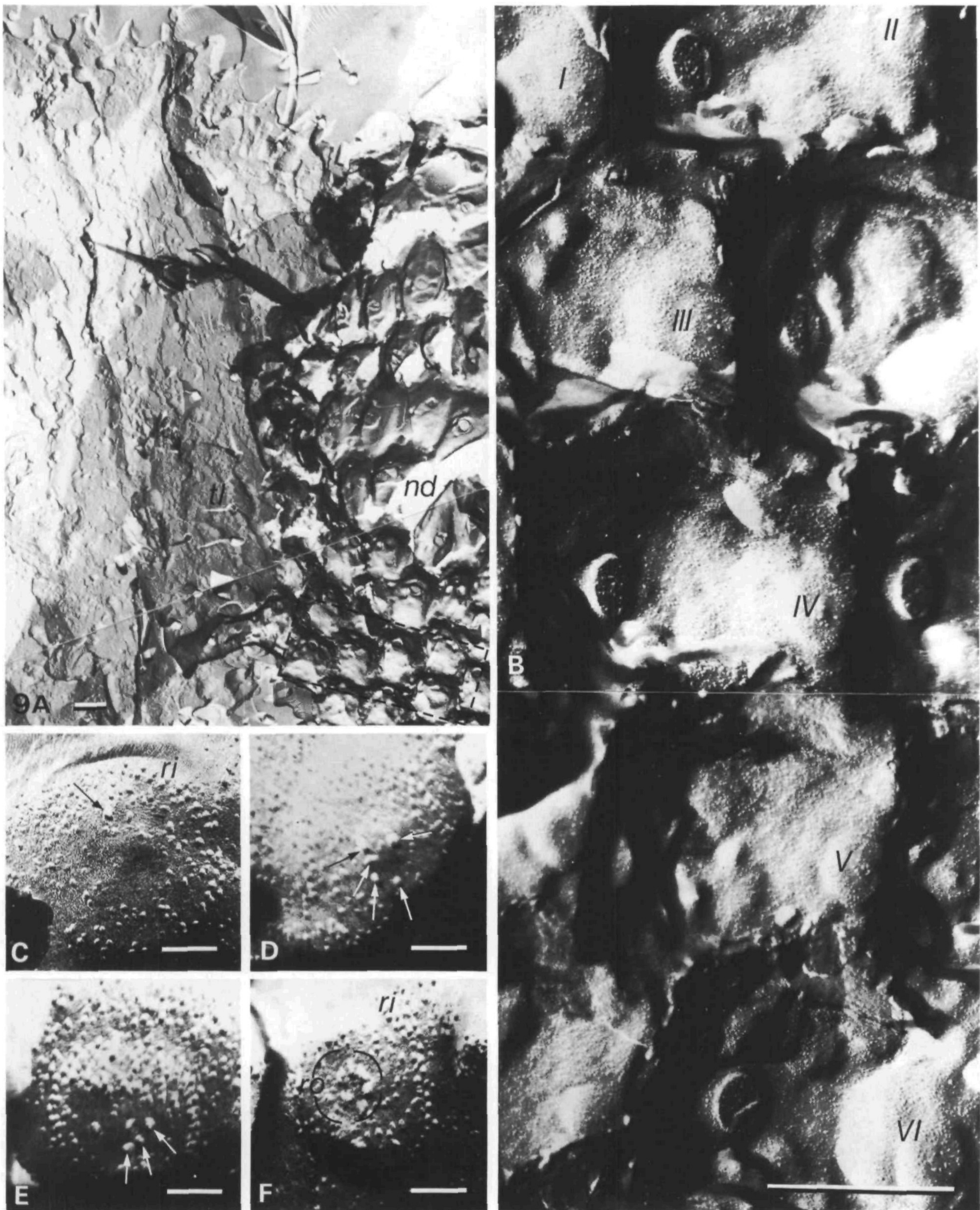

Fig. 9. Freeze-fracture replicas from *nd9-28°C/tl* conjugants. A. Survey, showing a trichocyst-free cell (*tl*) in cross-fracture and the fractured cell membrane of an *nd9-28°C* conjugant (*nd*). B. Detail from the *nd9-28°C* cell surface outlined in A, displaying several exocytosis sites (encircled by ≈300 nm large particle rings) with different stages of rosette assembly (*I–VI*). C–F. Possible assembly stages of rosettes (*ro*), within rings (*ri*); selected from different *nd9-28°C* conjugants; C, corresponds to the morphology typical of non-cured *nd9-28°C* (cf. Beisson *et al.* 1976); F, shows a rosette with six particles in an arrangement typical of wild-type cells (cf. Plattner, 1987). D,E. Possible stages between C and F, with partly scattered rosette particles (arrows). Note that these are larger (≈13 nm) than other particles (8–10 nm). Bars: A,B, 1 μm: C–F, 0·1 μm.

We do not know which mechanism accounts for the occasional occurrence of a small number of free trichocysts in late *tl* conjugants (Fig. 4D). All other phenomena referred to as 'secretory function repair' in this paper are most probably due to exchange of molecular components only, though their identity has not been established.

The assumption of a transfer of 'curing factors' by diffusion is based on the following: (1) transfer of micronuclei has not been observed within the time period of our analyses. (2) Intercellular connections, occurring during the time period of our analyses, between conjugating *Paramecium* cells, are rather narrow (<1 μm; Schneider, 1963) and such connections appear clogged by fibrillar, electron-dense materials (Schneider, 1963), which would act as a microfilter. (3) Concomitantly, organelles even smaller than trichocysts, such as erythromycin-resistant mitochondria, are not transferred during conjugation (Perasso & Adoutte, 1974). (4) In fact, only molecular components were shown to be transferred during conjugation in *Paramecium* (Berger, 1976) as in *Tetrahymena* cells (McDonald, 1966).

Finally, the use of *nd9-28°C* and *tl* mutants for conjugation experiments allowed us to establish that the repair of the secretory capacity is paralleled (or, possibly, caused) by the assembly of 'rosettes' at the predetermined secretory sites within the cell membrane. This supports similar conclusions inferred from ultrastructural analyses of different genotypes (Beisson *et al.* 1976, 1980; Satir, 1976; Pouphile *et al.* 1986) or of corresponding phenocopies (Pape & Plattner, 1985). (For reviews on these aspects, see Plattner (1987), and Adoutte (1988).) It also appears that 'connecting materials' between cell membrane and trichocysts are assembled during functional repair in *nd* cells (data not shown). Analyses of this type have not been possible with the strains used before.

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