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Transport of membrane receptors and the mechanics of sexual cell fusion in *Chlamydomonas eugametos*

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During sexual reproduction in the heterothallic, biflagellate, green alga *Chlamydomonas*, gametes adhere together via their agglutinins, sex-specific glycoproteins extrinsically bound to the flagellar membrane. Using an antibody specific for a *C. eugametos* agglutinin, we illustrate that agglutinins engaged in adhesion are transported to the flagellar tips. This tipping phenomenon, together with a particular orientation of the flagella, forms part of the mechanism by which gametes position themselves properly for fusion in pairs.

Sexual cell fusion; Membrane receptor transport; *(Chlamydomonas eugametos)*

1. INTRODUCTION

In many cases, during sexual reproduction, gametes fuse together via specialised areas on their cell surfaces. The unicellular green alga *Chlamydomonas eugametos* is a good example, because the cell body is permanently covered by a cell wall and gamete protoplasts can only fuse via minute papillae that just protrude through the cell wall between the bases of their two flagella. The flagella are not covered by a cell wall but by an extensive glycocalyx, that impedes direct fusion, but is instrumental in activating the outgrowth of the papillae in preparation for fusion [1–3]. A basic problem is to explain how free swimming mating partners, referred to as mating type plus and minus (mt^+ and mt^-), which initially make chance contact via their flagella, eventually bring their papillae in contact.

The glycocalyx of each gamete flagellum contains a large, linear sex-specific glycoprotein which is involved in sexual adhesion and has been demonstrated to contain the binding site for a ligand on the flagellar membrane of the sexual partner [4,5]. Like many other flagellar membrane components [6,7], the agglutinins are thought to be mobile in the flagellar membrane. More particularly, agglutinins involved in adhesion are thought to be transported to the flagellar tips during agglutination, for the sites of adhesion are seen to change during this process from random contacts to tip-to-tip contacts [8–10]. While the tipping of agglutinins has long been assumed, it has never been directly visualised. However, we have now labeled the mt^- agglutinin with a monoclonal antibody (Mab) to illustrate how the agglutinin becomes redistributed during sexual agglutination.

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Abbreviations: Mab, monoclonal antibody; mt, mating type; FITC, fluorescein isothiocyanate; TRITC, tetramethylrhodamine isothiocyanate

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A second flagellar phenomenon is also important for papillar contact, namely the orientation of the partner flagella. It will be shown that during sexual agglutination the flagella assume a particular orientation so that the papillae become positioned directly opposite each other.

2. MATERIALS AND METHODS

C. eugametos strains *Utex* 9 (mt^+) and *Utex* 10 (mt^-) from the Algal Collection at the University of Texas at Austin, USA, were cultivated in Petri dishes on an agar-containing medium as previously described [8]. Gametes were produced by flooding 2- to 3-week-old cultures with distilled water. mt^+ and mt^- gametes were mixed in equal concentrations to allow sexual agglutination to occur. Cells were fixed in 1.25% glutaraldehyde for most immunofluorescence studies, and in 4% formaldehyde/0.05% tannic acid, or 2% OsO_4, to maintain sexual contacts between agglutinating gametes. Cells were observed and photographed under a Zeiss photomicroscope or a Cambridge stereoscan electron microscope. For scanning electron microscopy, the procedure described by Mesland [8] was followed.

Two Mabs were used. One, referred to as Mab 44.2, specifically recognises epitopes containing the sugars 6-*O*-methyl mannose and 3-*O*-methyl glucose [11]. These sugars only occur in the mt^- gamete used in this study and thus the Mab was a specific label for the mt^- cells. The other antibody is referred to as Mab 66.3 and is specific for the mt^- agglutinin on mt^- gametes. However, it also recognises at least one mt^+ flagellar glycoprotein and therefore is not sex-specific. Consequently, in the immunofluorescence study presented here, it could only be used to monitor the distribution of the mt^- agglutinin in conjunction with Mab 44.2 with which the mt^- cells were identified. Mab 44.2 was directly labeled with TRITC and Mab 66.3 with FITC as described by Mishel and Shiigi [12].

3. RESULTS AND DISCUSSION

When mt^+ and mt^- gametes are mixed, the flagella make point contacts anywhere on their surfaces, yet just before cell fusion the partner flagella adhere over their entire length as illustrated in fig.1A. This automatically aligns the papillae which can be seen protruding out of the flagellar ridges. Two phenomena are important for alignment, viz. the tipping of agglutination contact sites and a particular orientation of the flagella. The flagella of each pair can be seen in fig.1A to be oriented around one of the cell bodies. It was recently shown [13] that this is the mt^- gamete body. Thus, the mt^- flagella can become completely reflexed around their own bodies, which is normally not observed. The orientation shown in fig.1A results in a face to face confrontation. In this way, the tips of both papillae become directed at each other and when mating is observed under the light microscope, the flagellar ridges seemed to be buffeted together, suggesting that the papillae are physically forced into each other. To understand the importance of this orientation, consider an alternative orientation, which we have never observed, in which both gametes hold their flagella forward like mt^+ gametes, the flagella could still adhere over most of their length, but the cells would not lie vis-à-vis but side by side, and consequently the papillae would seldom if ever make contact.

In order to test whether agglutinins are transported to the flagellar tips during agglutination, gametes were mixed and at subsequent intervals fixed with glutaraldehyde to visualise the distribution of the mt^- agglutinin using Mab 66.3, labeled directly with FITC. The 3-dimensional clumps were broken up by fixation with glutaraldehyde in order to mount the single cells on a glass slide so that many flagella could be photographed in a single focal plane. In these cells it was shown that before agglutination, the mt^- agglutinin was evenly distributed over the flagella, but in samples taken at subsequent intervals, the agglutinins became concentrated at the tips of many flagella. It was not expected that tipping of agglutinins would involve all gamete flagella in one sample because agglutination does not progress synchronously, some cells fusing in 5 min and others only after 60 min [8]. Several lines of evidence indicate that gametes tip their agglutinins as a direct result of the interaction with the opposite mating type. (i) Cell-free culture supernatant derived from partner gametes did not induce tipping. (ii) When aggregates of agglutinating gametes were fixed intact by using formaldehyde/tannic acid, the tipped agglutinins

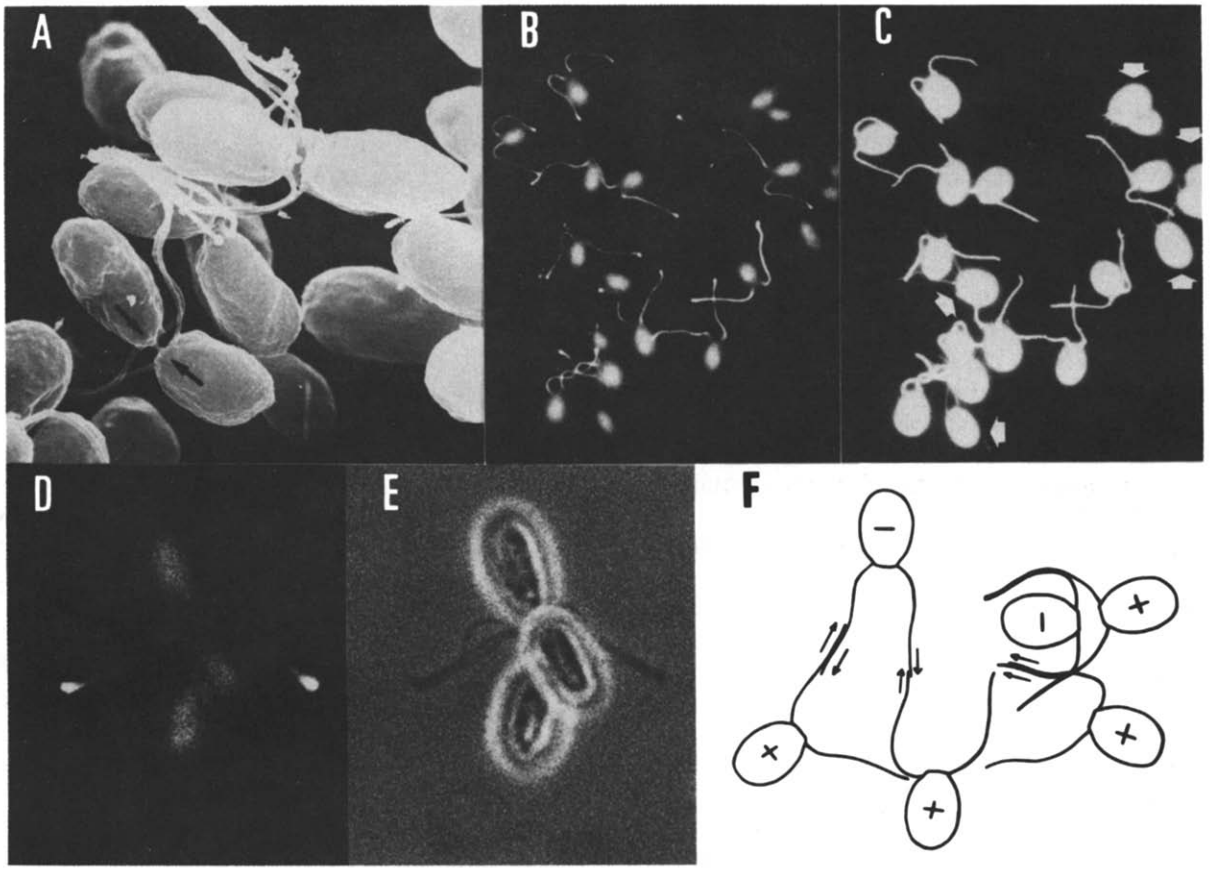

Fig.1. (A) Scanning electron micrograph of agglutinating gametes just before cell fusion. Papillae are indicated by arrows. (B,C) Fluorescence micrographs of gametes fixed with glutaraldehyde during sexual agglutination showing the mt^- gametes labeled with Mab 44.2-TRITC (C) and the distribution of the mt^- agglutinin over the flagella labeled with Mab 66.3-FITC (B). The mt^+ gametes are indicated by arrows. (D,E) Micrographs of agglutinating gametes fixed with formaldehyde/tannic acid illustrating the position of partner flagella adhered over their entire lengths (E, phase contrast) and the distribution of antigens (including the mt^- agglutinin) recognised by Mab 66.3-FITC (D). (F) Diagram illustrating how tipping helps gametes sort themselves out into pairs.

were detected at those points where the tips of several flagella adhered together (fig.1D,E). (iii) After cell fusion, when the gamete flagella deagglutinated, tipping also disappeared. (iv) When cell fusion was prevented by treating agglutinating gametes with 1 mM cysteine (Samson, personal communication), the gametes remained agglutinating for at least an hour and then practically all of the mt^- gametes could be seen to have tipped their agglutinins (fig.1B,C). Note that many of the mt^- gametes maintained the reflexed flagellar orientation typical of the advanced stage of agglutination shown in fig.1A and E. Furthermore, fig.1B and C clearly shows that the mt^+ antigens recognised by Mab 66.3 were also tipped, while those mt^- antigens recognised by Mab 44.2 were not. Since several major flagellar glycoproteins express these latter antigenic sites [11], this indicates that tipping does not represent a mass flow of glycoproteins to the tips, but rather the specific transport of a few components, including the agglutinins. The following experiment demonstrates in a direct way that agglutinins are mobile in the plane of the membrane and can be redistributed to the flagellar tips. When living mt^- gametes were treated with Mab 66.3 (labeled with FITC) and fixed immediately, the label was distributed over the whole flagellum. When fixed 30 s after mixing,

the label was found in small patches over the entire length of the flagellar surface, while if cells were left for more than 5 min before fixation, the label became concentrated at the tips. Apparently, in this experiment, the agglutinin was patched and drawn to the flagellar tips by the cross-linking action of the antibody (comparable to the 'capping' process in lymphocytes). Although many mt^- agglutinin molecules became concentrated at the tips during sexual agglutination, a portion was distributed over the entire flagellum, for the subapical part of the flagellum did not loose its fluorescence altogether (fig.1B). We can rationalize this by assuming that this ensures that the complete flagellar surface remains agglutinable. How the tipping of some, but not all agglutinins is achieved, is unknown.

Tipping is not only important for partner gametes to be able to fuse, but also for the first stages of agglutination when partners have to sort themselves out from other gametes that co-agglutinate. A simple example is represented in fig.1F, where two gametes that adhere via their flagella tip-to-base will, as a result of tipping, become joined by their tips only, which is a labile contact compared to flagella that adhere over their entire length. In practice, tip-to-base contacts are frequently seen in the early stages of sexual agglutination, but after about 6 min, tip-to-tip contacts predominate [8]. This stage has been referred to as tip-locking [14] and in *C. eugametos* allows the rest of the aligned flagella to become adhered along their entire lengths. Tipping in *Chlamydomonas* is analogous to the much better known capping of membrane receptors in lymphocytes [15], but it is the only example we know of that has such dramatic morphological consequences. Various aspects of transport have been described and transport of agglutinins has been invoked as explaining any necessary realignment of flagellar tips during sexual agglutination [10–14,16]. However, this is the first time that the general distribution of a *Chlamydomonas* agglutinin has been visualised, and unequivocally demonstrated to accumulate at the flagellar tips during agglutination.

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