Calcium influx regulates antibody-induced glycoprotein movements within the *Chlamydomonas* flagellar membrane

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Summary

The *Chlamydomonas* flagellar surface exhibits a number of dynamic membrane phenomena associated with whole-cell gliding locomotion and the early events in fertilization. Crosslinking of a specific population of flagellar surface-exposed glycoproteins with the lectin concanavalin A or an anti-carbohydrate mouse monoclonal antibody, designated FMG-1, results in a characteristic pattern of glycoprotein redistribution within the plane of the flagellar membrane. Recent evidence suggests that flagellar membrane glycoprotein movements are associated with both whole-cell gliding motility and the early events in mating. It is of interest to determine the transmembrane signaling pathway whereby crosslinking of the external domains of flagellar glycoproteins activates the intraflagellar machinery responsible for translocation of flagellar membrane glycoproteins.

The redistribution of flagellar membrane glycoproteins requires micromolar levels of free calcium in the medium; lowering the free calcium concentration to 10^{-7} M results in complete but reversible inhibition of redistribution. Redistribution is maximal in the presence of 20 μM free calcium in the medium. Redistribution is inhibited in the presence of 20 μM free calcium by the calmodulin antagonists trifluoperazine, W-7 and calmidazolium, the calcium channel blockers diltiazem, methoxyverapamil (D-600) and barium chloride, and the local anesthetics, lidocaine and procaine. The actions of all of these agents can be interpreted in terms of a requirement for calcium in the signaling mechanism associated with flagellar glycoprotein redistribution. In particular, the requirement for micromolar calcium in the external medium and the effects of specific calcium channel blockers suggest that flagellar membrane glycoprotein crosslinking may induce an increase in calcium influx, which may be the initial trigger for activating the flagellar machinery responsible for active movement of flagellar membrane glycoproteins.

Key words: calcium, flagella, membrane, glycoprotein, *Chlamydomonas*, signal transduction, cell surface.

Introduction

Eukaryotic cells exercise control over the distribution of plasma membrane proteins; they can maintain asymmetric distributions of cell surface proteins and can actively change the distribution of proteins within the plane of the plasma membrane. In the case of the *Chlamydomonas* flagellar membrane, crosslinking of surface-exposed flagellar membrane glycoproteins by multivalent ligands (antibodies or lectins) results in a global redistribution of the population of proteins recognized by the multivalent ligand within the plane of the plasma membrane in a very characteristic pattern (Goodenough and Jurivich, 1978; Bloodgood *et al.* 1986; Homan *et al.* 1988; Kooijman *et al.* 1989). The active movement of flagellar membrane glycoproteins appears to be involved in the normal physiological processes of whole-cell gliding motility (Bloodgood and Salomonsky, 1989*a*) and fertilization (Homan *et al.* 1987). It is presumed that crosslinking of surface-exposed portions of the flagellar membrane glycoproteins (due to antibodies or lectins in the experimental situation or due to contact with a solid substratum or the surface of a gametic flagellum in the physiologically relevant situations) initiates a transmembrane signaling pathway that results in activation of the motile machinery for movement of the glycoproteins within the plane of the membrane (Bloodgood, 1989). In *Chlamydomonas*, calcium has been implicated in the regulation of flagellar stability (Lefebvre *et al.* 1978; Quader *et al.* 1978), flagellar motility (Hyams and Borisy, 1978; Bessen *et al.* 1980), phototaxis (Dolle *et al.* 1987), mating events (Snell *et al.* 1982; Bloodgood and Levin, 1983; Kaska *et al.* 1985), and microsphere movements along the flagellar membrane (Bloodgood *et al.* 1979). For the first time, we report here on the involvement of calcium in the regulation of the transmembrane signaling events associated with flagellar glycoprotein redistribution.

Materials and methods

Cell strains and reagents

Unless otherwise indicated, all experiments have been performed on vegetative cells of *Chlamydomonas reinhardtii* strain pf-18

grown in Medium I of Sager and Granick (1953) at 21 °C using a light/dark cycle of 14 h light and 10 h dark. Cultures were harvested after 3–4 days of growth at a cell concentration of approximately 2×10^6 to 3×10^6 cells ml^{-1}. For induction of gametogenesis, vegetatively grown cells were washed into nitrogen-free growth medium and exposed to continuous light for 15 h.

The mouse hybridoma producing the FMG-1 anti-carbohydrate mouse monoclonal antibody was obtained as previously described (Bloodgood *et al.* 1986). In order to obtain large amounts of monoclonal antibody, hybridoma cells were washed into phosphate-buffered saline (PBS) and injected intraperitoneally into Balb/C mice; ascites fluid was collected and the IgG fraction purified using the Affi-Gel Protein A MAPS procedure (BioRad Laboratories, Richmond, CA). Purified monoclonal antibodies were conjugated to fluorescein using a 1:60 mass ratio of fluorescein isothiocyanate to mouse monoclonal IgG in 0.2 M bicarbonate buffer, pH 9.5, for 60 min at 37 °C. This mixture was chromatographed over Sephadex G-50 (Pharmacia Fine Chemicals) to remove unconjugated FITC and the labeled antibody was bound to a Whatman DE52 (Whatman Ltd) column equilibrated with 10 mM potassium phosphate, pH 7.5; antibody fractions with different levels of conjugation were eluted with potassium chloride, dialyzed against phosphate-buffered saline, pH 7.2, and concentrated using Centricon 30 microconcentrators (Amicon Corporation). Antibodies used for cell labeling and redistribution studies had a coupling ratio of 3–5 FITC molecules per IgG molecule. Protein concentration was determined by the method of Schacterle and Pollack (1973). Stock labeled antibody stored at 4 °C was diluted and then dialyzed for 2 h against the appropriate buffer just prior to use. FITC–concanavalin A was obtained from Vector Laboratories and dialyzed against the appropriate buffer for 2 h before use. W-5 and W-7 were purchased from Seikagaku America (St Petersburg, FL); all other reagents were obtained from Sigma Chemical Company (St Louis, MO). Stock solutions of ethylene glycol-bis(β-aminoethyl ether)-*N,N,N,N'*-tetraacetic acid (EGTA), calcium chloride, trifluoperazine, W-5, W-7, D-600 (methoxyverapamil), procaine–HCl and diltiazem–HCl were prepared in water; stock solutions were stored at −20 °C in the dark. Lidocaine was dissolved in a small volume of 1 M HCl and then titrated to pH 6.0 with NaOH.

Assay for flagellar membrane glycoprotein redistribution

The general features of antibody and concanavalin A-induced redistribution of flagellar membrane glycoproteins have been described (Bloodgood *et al.* 1986). Except where otherwise stated, the FMG-1 anti-carbohydrate mouse monoclonal antibody was utilized to stimulate flagellar membrane glycoprotein redistribution; the preparation and characteristics of this monoclonal antibody have been described previously (Bloodgood *et al.* 1986). Although certain experiments (see Figs 1, 7, 8) were performed in *Chlamydomonas* growth medium (Medium I of Sager and Granick, 1953), most of the redistribution experiments were performed in 10 mM potassium phosphate buffer, pH 6.8–7.0. In general, the cells in ice-cold 10 mM potassium phosphate buffer, pH 6.8–7.0, were mixed with the appropriate concentrations of EGTA, calcium chloride and the particular drug to be tested at a cell concentration of 2×10^7 cells ml^{-1}. After an initial 5- to 10-min period of incubation on ice, an equal volume of FITC-derivatized FMG-1 monoclonal antibody (containing the appropriate drug, EGTA and calcium concentrations) was added to the cells, giving a final antibody concentration of 100 μg ml^{-1} and a cell concentration of 1×10^7 cells ml^{-1}; the mixture was then incubated for an additional 10 min at 0–4 °C. Redistribution was initiated by rapidly warming the samples in a 27 °C water bath where the cells were incubated for 12–15 min with intermittent shaking. Redistribution was terminated by mixing the cells with an equal volume of 2% glutaraldehyde in 10 mM potassium phosphate buffer, pH 6.8–7.0. In some experiments FITC–concanavalin A (at a concn of 100 μg ml^{-1}) was substituted for the FITC–FMG-1 monoclonal antibody. Since all of the redistribution was induced by crosslinking flagellar surface-exposed proteins using either a lectin (concanavalin A) or an anti-carbohydrate monoclonal antibody (FMG-1), the redistribution that is observed is specific for glycoproteins. In certain experiments gametic cells were substituted for vegetative cells.

Fixed samples were scored in a Zeiss Std 16 microscope equipped with epifluorescence optics for the extent of antibody or lectin redistribution. A minimum of 200 cells from each sample were scored as to being at one of five arbitrary stages in the redistribution process (see the illustrations on the abscissa in Figs 1–8; and Bloodgood *et al.* 1986); these data were plotted as a frequency distribution for the population, indicating how far the population had been redistributed over the 12- to 15-min period of incubation at 27 °C. The stages of redistribution, as presented in the figures from left to right, are known to be in correct temporal sequence (Bloodgood *et al.* 1986). In addition, it has been demonstrated that antibody redistribution reflects a parallel redistribution of the principal concanavalin A binding glycoproteins of the flagellar membrane (Bloodgood *et al.* 1986). Data in Figs 1–6 are presented as bar graphs; although the data in Figs 7 and 8 are presented as line graphs, an identical experimental protocol was followed. In all of the experiments, the percentage of flagellated cells at the end of the redistribution period was determined as an index of cell health and of toxicity of the drug concentrations being utilized.

The free calcium concentration in the EGTA–calcium chloride mixtures was calculated using the methods of Portzehl *et al.* (1964).

Results

The major cell surface-exposed protein in the *Chlamydomonas* flagellar membrane is a concanavalin A-binding glycoprotein of 350 000 apparent molecular weight (Bloodgood and Workman, 1984; Bloodgood, 1987). Crosslinking of the flagellar surface with an anti-carbohydrate monoclonal antibody recognizing this glycoprotein results in a dramatic and characteristic pattern of redistribution of the population of this flagellar protein (Bloodgood *et al.* 1986). This antibody-induced redistribution is sensitive to the free calcium concentration in the external medium (Figs 1, 2). Redistribution occurs normally in cell growth

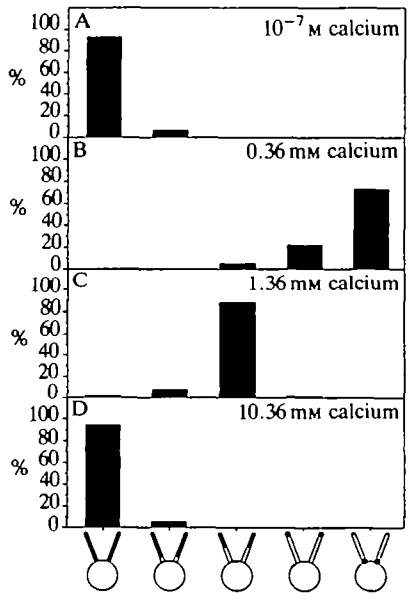

Fig. 1. Effect of a wide range of external total calcium concentrations on antibody-induced flagellar glycoprotein redistribution. Experiment performed in growth medium. A. $<10^{-7}$ M free calcium (2 mM EGTA plus 0.36 mM calcium chloride); B, 0.36 mM calcium; C, 1.36 mM calcium; D, 10.36 mM calcium. Both very low and very high calcium concentrations inhibit redistribution.

medium (Medium I of Sager and Granick, 1953), which contains 0.36 mM total calcium (although the free calcium concentration is considerably lower because of the presence of 1.7 mM sodium citrate); in this medium, redistribution is maximal in terms of rate and extent (Fig. 1; and Bloodgood *et al.* 1986). If an additional 1 mM of calcium chloride is added to this medium, glycoprotein redistribution is significantly inhibited; if 10 mM calcium chloride is added, then glycoprotein redistribution is completely inhibited (Fig. 1). The effect of reducing the free calcium concentration in the medium was observed using a medium containing only 10 mM potassium phosphate buffer, pH 6.8, 1 mM EGTA, and varying concentrations of total added calcium chloride (Fig. 2). Maximal redistribution occurred at 2×10^{-5} M free calcium; some inhibition of redistribution was observed at 2×10^{-6} M free calcium concentration. Significant inhibition of redistribution, with cells in the population showing all intermediate stages in the redistribution process, was observed at 7×10^{-7} M free calcium concentration; total inhibition of redistribution occurred at 10^{-7} M free calcium concentration (Fig. 2). These observations suggest that micromolar concentrations of free calcium in the medium are necessary in order for *Chlamydomonas* to respond to antibody crosslinking of the flagellar surface by activating intraflagellar machinery for redistribution of proteins in the plane of the flagellar membrane. Similar results were obtained for concanavalin A-induced flagellar membrane glycoprotein redistribution (data not shown).

In order to determine whether calcium fluxes across the flagellar membrane accompanied (and/or were required for) the antibody-induced redistribution of flagellar membrane glycoproteins, the effect of the calcium channel

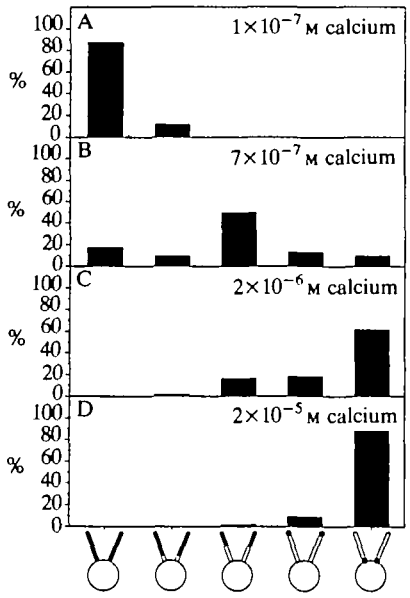

Fig. 2. Effect of free calcium concentration on antibody-induced flagellar glycoprotein redistribution. Experiment performed in 10 mM potassium phosphate buffer, pH 6.8, using 1 mM EGTA and varying concentrations of calcium chloride. A. 1×10^{-7} M free calcium; B, 7×10^{-7} M free calcium; C, 2×10^{-6} M free calcium; D, 2×10^{-5} M free calcium. 10^{-7} M free calcium does not permit redistribution to occur while 20 μM free calcium allows maximal redistribution; 0.7 μM and 2 μM concentrations of free calcium allow an intermediate amount of redistribution to occur.

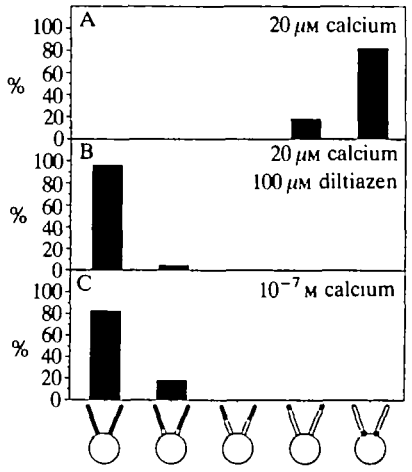

Fig. 3. Effect of the calcium channel blocker diltiazem on antibody-induced flagellar glycoprotein redistribution. A. 20 μM free calcium; B, 100 μM Diltiazem in the presence of 20 μM free calcium; C, 10^{-7} M free calcium. Diltiazem at this concentration was totally effective in blocking flagellar glycoprotein redistribution in the presence of 20 μM free calcium, a calcium concentration normally permissive for redistribution.

blockers D-600 (methoxyverapamil), diltiazem and barium chloride on flagellar glycoprotein redistribution was determined. These calcium channel blockers are known to inhibit phototaxis and the photophobic reaction in *Chlamydomonas* (Nultsch *et al.* 1986; Schmidt and Eckert, 1976); furthermore, the *Chlamydomonas* flagellar membrane is known to possess binding sites for diltiazem (Dolle and Nultsch, 1988). Diltiazem and D-600 both inhibited flagellar membrane protein redistribution in a dose-dependent manner (Figs 3, 4); 100 μM diltiazen (in the presence of 20 μM free calcium) almost completely inhibited flagellar glycoprotein redistribution and mimiced the effect of lowering the free calcium concentration in the medium to 10^{-7} M (Fig. 3). While concentrations of 50–100 μM D-600 were partially inhibitory, concentrations of D-600 of 200 μM and above completely inhibited flagellar membrane protein redistribution (Fig. 4). Barium chloride at concentrations of 10 mM or higher effectively inhibited flagellar glycoprotein redistribution in the presence of 20 μM free calcium (data not shown).

The local anesthetics lidocaine and procaine inhibit fast sodium channels at physiologically effective (anesthetic) doses (Strichartz and Ritchie, 1987) but at higher concentrations can be used as tools to inhibit slow calcium channels (Coyle and Sperelakis, 1987; Spedding and Berg, 1985). Both lidocaine and procaine inhibit flagellar glycoprotein redistribution at similar concentrations, although lidocaine is consistently somewhat more potent (Fig. 5); the inhibitory effect is substantial at 5 mM and virtually complete at 14 mM lidocaine or procaine (Fig. 5). At a free calcium concentration of 20 μM (1 mM EGTA, 1 mM $CaCl_2$), 14 mM lidocaine is almost completely inhibitory and mimics the effect of reducing the free calcium concentration to 10^{-7} M in the absence of the drug. If the free calcium concentration is raised to almost mM level (1 mM EGTA, 2 mM $CaCl_2$), the same concentration of lidocaine does not exhibit an inhibitory effect and the flagellar membrane redistribution resembles that normally occurring at the 20 μM free calcium level (Fig. 6).

In order to ask whether the calcium requirement might be acting through calmodulin, which is present in high

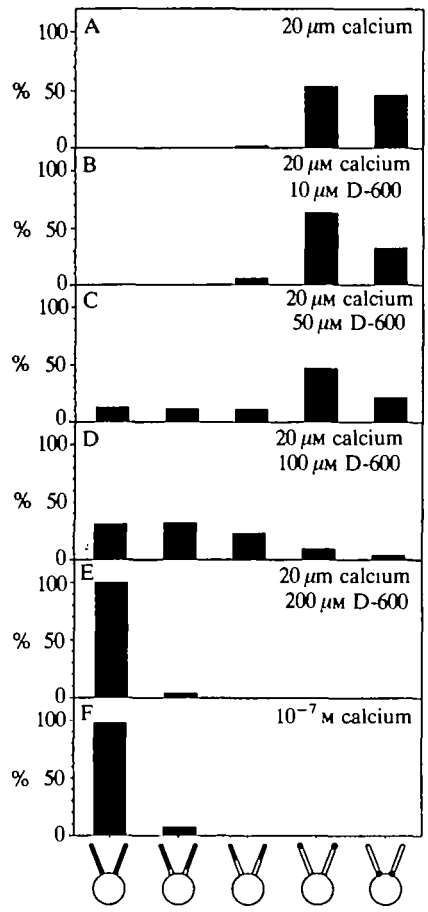

Fig. 4. Effect of the calcium channel blocker D-600 (methoxyverapamil) on antibody-induced flagellar glycoprotein redistribution. A. 20 μM free calcium; B, 10 μM D-600 with 20 μM free calcium; C, 50 μM D-600 with 20 μM free calcium; D, 100 μM D-600 with 20 μM free calcium; E, 200 μM D-600 with 20 μM free calcium; F, 10^{-7} M free calcium. Increasing concentrations of D-600 progressively inhibit redistribution; 200 μM D-600 in the presence of 20 μM free calcium exhibits the same degree of inhibition as 10^{-7} M free calcium alone.

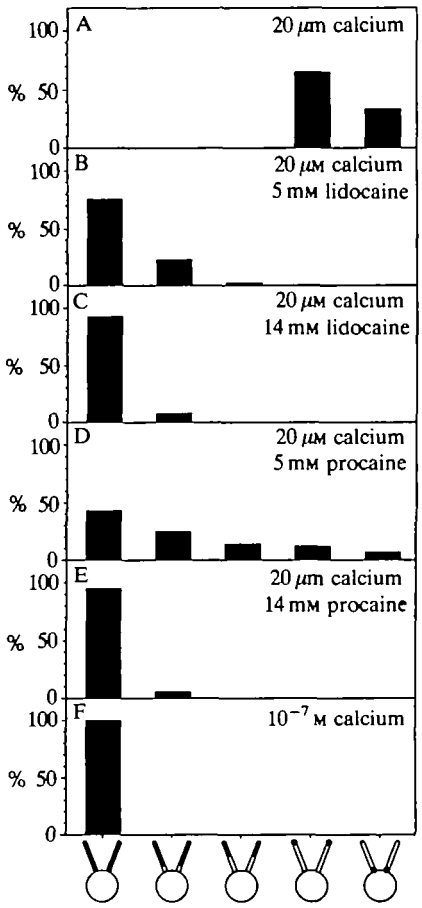

Fig. 5. Effect of lidocaine and procaine on antibody-induced flagellar glycoprotein redistribution. A. 20 μM free calcium; B, 5 mM lidocaine with 20 μM free calcium; C, 14 mM lidocaine with 20 μM free calcium; D, 5 mM procaine with 20 μM free calcium; E, 14 mM procaine with 20 μM free calcium; F, 10^{-7} M free calcium. At a concentration of 5 mM, lidocaine is a more effective inhibitor than procaine; at 14 μM both lidocaine and procaine exhibit almost complete inhibition, comparable to the situation observed in 10^{-7} M free calcium alone.

concentrations in the *Chlamydomonas* flagellum (Gitelman and Witman, 1980), the effect of various calmodulin antagonists was observed. Trifluoperazine (TFP) inhibited flagellar glycoprotein redistribution in a dose-dependent manner with maximal inhibition occurring at about 14–18 μM (Fig. 7). The trifluoperazine effects were fully reversed after washing out the drug. Trifluoperazine is equally effective at inhibiting the flagellar membrane redistribution induced by cross-linking with concanavalin A or with the FMG-1 mouse monoclonal antibody. Trifluoperazine inhibits redistribution in both gametic and vegetative cells at similar drug concentrations (data not shown). The calmodulin antagonist W-7 also reversibly inhibited flagellar glycoprotein redistribution at concentrations similar to those for TFP (Fig. 8); 18 μM W-7 was very effective at inhibiting redistribution while 18 μM W-5, the much less potent analog (Hidaka and Tanaka, 1982), left the cells unaffected (Fig. 8). The W-7 effect was reversed completely by washing the cells free from the drug (Fig. 8). A third drug that is known to be a calmodulin antagonist (R24571 or calmidazolium) was also tested for its effect on flagellar glycoprotein redistribution with somewhat different results; at concentrations of 1 μM and higher, this drug blocked all of the cells in the population at an intermediate stage in redistribution equivalent to the third (or middle) pattern illustrated on the abscissa in Figs 7 and 8. The effective doses of these drugs were observed to be cell density-dependent, as was shown by Detmers and Condeelis (1986) for gametic cells of *C. reinhardtii*.

Discussion

Calcium regulates many cellular activities that are associated with the flagella of *Chlamydomonas reinhardtii*: the nature of the flagellar waveform (Hyams and Borisy, 1978; Bessen *et al.* 1980), flagellar stability (Lefebvre *et al.* 1978; Quader *et al.* 1978), flagellar surface motility (Bloodgood *et al.* 1979), phototaxis (Dolle *et al.* 1987) and the photophobic response (Schmidt and Eckert, 1976). A number of observations also suggest some role for calcium in the early (flagellar) events occurring during mating in *Chlamydomonas* (Bloodgood and Levin, 1983; Snell *et al.* 1982; Kaska *et al.* 1985; Detmers and Condeelis, 1986).

The present report demonstrates for the first time that an additional *Chlamydomonas* flagella-associated

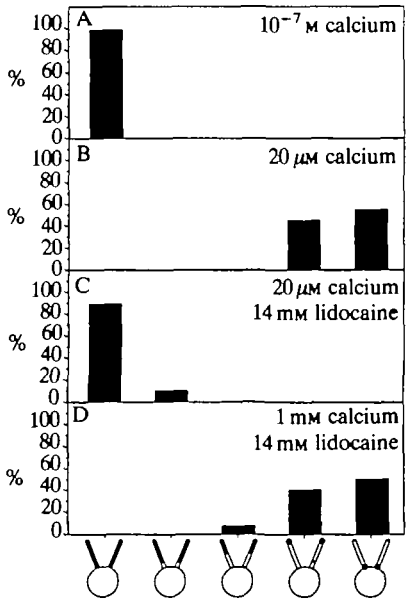

Fig. 6. Calcium reversal of lidocaine inhibition. A. 10^{-7} M free calcium; B, 20 μM free calcium; C, 14 mM lidocaine in the presence of 20 μM free calcium (1 mM EGTA, 1 mM calcium); D, 14 mM lidocaine in the presence of approximately 1 mM free calcium (1 mM EGTA, 2 mM calcium). Lidocaine in the presence of 20 μM free calcium resembles the situation in 10^{-7} M free calcium alone (almost total inhibition); lidocaine in the presence of 1 mM free calcium resembles the situation in 20 μM free calcium alone (almost maximal redistribution).

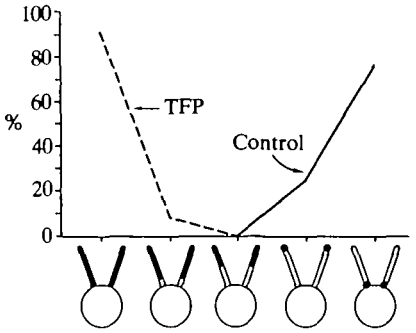

Fig. 7. Effect of 16 μM trifluoperazine (TFP) on antibody-induced flagellar glycoprotein redistribution. This concentration of TFP completely and reversibly inhibited antibody redistribution.

phenomenon, the redistribution of flagellar membrane glycoproteins induced by crosslinking with antibodies or lectins (Goodenough and Jurivich, 1978; Bloodgood *et al.* 1986; Homan *et al.* 1988; Kooijman *et al.* 1989), is regulated by calcium and, in particular, by calcium influx from the extracellular medium. This conclusion rests on four types of treatments that inhibit the normally observed induction of flagellar glycoprotein redistribution by crosslinking with antibodies or concanavalin A: (1) lowering the free calcium concentration in the medium to 10^{-7} M; (2) specific calcium channel blockers (diltiazem, D-600 (methoxyverapamil), barium chloride); (3) calmodulin antagonists (TFP, W-7, calmidazolium); and (4) local anesthetic amines (lidocaine and procaine). The one common denominator for all of these treatments is an association with calcium.

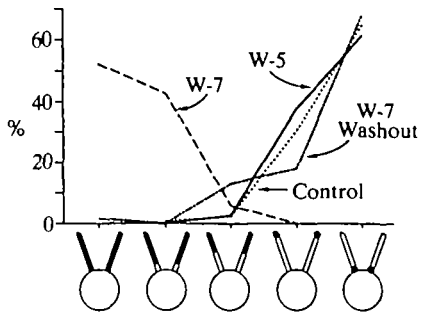

Fig. 8. Effect of W-5 and W-7 on antibody-induced flagellar glycoprotein redistribution. W-7 (18 μM) inhibited antibody redistribution and this inhibition was reversed by removing the cells from the drug prior to warming. W-5 at the same concentration (18 μM) did not inhibit the redistribution.

The agents diltiazem and D-600 (methoxyverapamil) are known to be specific calcium channel blockers (Triggle, 1982; Godfraind *et al.* 1986). Diltiazem inhibits phototaxis in *C. reinhardtii* (Nultsch *et al.* 1986) and the flagellar membrane of this species has specific binding sites for diltiazem (Dolle and Nultsch, 1988). D-600 (methoxyverapamil) inhibits light-induced backward swimming (the photophobic effect) in *C. reinhardtii* (Schmidt and Eckert, 1976), an event known to involve calcium.

Although the primary pharmacological and anesthetic effect of the local anesthetic amines lidocaine and procaine is to block fast sodium channels (Strichartz and Ritchie, 1987), these agents are known to block, at higher concentrations, slow calcium channels (Spedding and Berg, 1985; Coyle and Sperelakis, 1987) and their cellular actions often mimic the action of the specific calcium channel blockers (Huwyler *et al.* 1985; Silva *et al.* 1987). Elevation of the extracellular calcium concentration counteracts the effects of lidocaine observed in the present study and also the effects of lidocaine on mating-associated events in *C. reinhardtii* (Snell *et al.* 1982). Schmidt and Eckert (1976) observed that procaine inhibited light-induced flagellar reversal in *C. reinhardtii* and interpreted procaine's effect as blocking calcium channels.

Trifluoperazine (TFP), W-7 and calmidazolium are calmodulin antagonists and can interfere with enzymes that are regulated by calcium and calmodulin. Calmodulin is present in the *Chlamydomonas reinhardtii* flagellum in high concentrations and much of the flagellar calmodulin is found in a non-ionic detergent extract of flagella (Gitelman and Witman, 1980). However, TFP, W-7 and calmidazolium have been reported to have a number of calmodulin-independent effects, primarily relating to their ability to interact with phospholipids (Landry *et al.* 1981; Roufogalis, 1982). All three drugs have been reported to inhibit protein kinase C (Schatzman *et al.* 1981, 1983; Tanaka *et al.* 1982; Wise *et al.* 1982; Mazzei *et al.* 1984). W-7 directly blocks voltage-dependent calcium channels in the ciliary membrane of *Paramecium* (Hennessey and Kung, 1984; Ehrlich *et al.* 1988).

All of the agents and conditions reported to inhibit flagellar membrane glycoprotein movements could be interpreted as having their effect by interfering with calcium fluxes into the cell. On the other hand, the different agents used as inhibitors in this study could be acting at several different levels: blocking calcium channels (diltiazem, W-7, barium chloride, lidocaine, procaine), inhibiting a calmodulin-regulated enzyme (TFP, W-7,

calmidazolium), altering the status of membrane phospholipids (TFP, lidocaine, procaine), or inhibiting protein kinase C (TFP, W-7, calmidazolium). Nevertheless, the inhibitory effect of these agents still collectively supports the conclusion that calcium plays an important role in the signal transduction pathway that regulates flagellar membrane glycoprotein redistribution in *Chlamydomonas*.

There are two alternative ways of interpreting the calcium dependence of flagellar glycoprotein redistribution. It could be that normally the intraflagellar free calcium level is permissive for the signaling events induced by membrane protein crosslinking; in this case, lowering the extracellular calcium or the presence of calcium channel blockers could be acting to allow the normal intraflagellar free calcium level to fall beneath some threshold level, due to the pumping of calcium out of the cell or into cytoplasmic compartments. The other interpretation, which we favor, is that flagellar membrane crosslinking induces an increased rate of influx of calcium, raising the intraflagellar free calcium level high enough to allow signaling events to activate the machinery for moving glycoproteins within the plane of the flagellar membrane.

Recent observations from this laboratory suggest that calcium may be functioning to regulate protein phosphorylation and dephosphorylation in *C. reinhardtii* flagella (Bloodgood and Salomonsky, 1989*b*). The protein kinase inhibitors H-7, H-8 and staurosporine (Hidaka *et al.* 1984) inhibit antibody-induced redistribution of flagellar membrane glycoproteins (Bloodgood and Salomonsky, 1989*b*). Recent experiments have demonstrated calcium-stimulated phosphorylation and dephosphorylation in Nonidet P-40 extracts of *C. reinhardtii* flagella (Bloodgood and Salomonsky, 1989*b*; Bloodgood, unpublished data).

Energy-dependent redistribution of populations of plasma membrane proteins is a widespread phenomenon (Taylor *et al.* 1971; Bourguignon and Bourguignon, 1984; Bourguignon, 1988; Trimmer and Vacquier, 1988; Myles and Primakoff, 1984). The role of calcium in plasma membrane protein redistribution in mammalian cells is not entirely clear. Although the crosslinking of cell surface proteins with antibodies or concanavalin A can trigger a transient increase in the intracellular free calcium level in a number of cell types (Freedman *et al.* 1975) depending on the particular ligand and cell type involved, most workers agree that extracellular calcium is not necessary for capping in most mammalian cells (Taylor *et al.* 1971; Brohee *et al.* 1985; Braun *et al.* 1979; Pozzan *et al.* 1982; Schreiner and Unanue, 1976; Salisbury *et al.* 1981) and even an intracellular increase in calcium has been shown to be unnecessary for successful capping in at least some systems (Majercik and Bourguignon, 1985; Nachshen *et al.* 1986). Other workers have argued for a causal relationship between the intracellular calcium level and capping (Bourguignon and Kerrick, 1983; Bourguignon, 1988; Grewal *et al.* 1987). In agreement with the results of the present report on glycoprotein redistribution in the flagellar membrane, receptor capping on lymphocytes has been reported to be inhibited by W-7 (Nelson *et al.* 1982), TFP (Ryan *et al.* 1974; Bourguignon and Balazovich, 1980), calmidizolium (Nelson *et al.* 1982), lidocaine (Ryan *et al.* 1974; Poste *et al.* 1975) and procaine (Poste *et al.* 1975), generally at concentrations very similar to those that were found to be effective on *Chlamydomonas*.

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