Cyclic AMP is one of the intracellular signals during the mating of *Chlamydomonas eugametos*

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Abstract. *Chlamydomonas eugametos* gametes of opposite mating type make cell-cell contact via their flagellar surfaces. This contact triggers an increase in the intracellular level of cyclic AMP (cAMP) and several cellular responses which are necessary for cell fusion. Here, we show that wheat-germ agglutinin, which binds to the flagellar surface and induces all mating responses, also increased the intracellular cAMP level. Dibutyryl-cAMP added to non-mating gametes induced flagellar twitching, cell-wall lysis, mating-structure activation, flagellar-tip activation and an increase in agglutinability. It did not induce agglutinin transport to the flagellar tip (tipping) and may not be the direct cause of flagellar twitching and flagellar-tip activation. In non-illuminated cells, dibutyryl-cAMP was far more effective in evoking mating reactions than in illuminated cells. Light induced a 50% decrease in the cAMP level within 1 min. Adenylate cyclase was found to be associated with cell membranes but only 8% of the total was present in the gamete flagella.

Key words: Cyclic AMP – Cell recognition – *Chlamydomonas* (gametes) – Mating responses – Plant cell signalling – Wheat germ agglutinin

Introduction

Sexual reproduction in the green alga *Chlamydomonas eugametos* is initiated when the flagella of mating-type-plus (*mt*+) gametes bind to those of mating-type-minus (*mt*−) gametes. Large aggregates of cells are formed (agglutination) in which cells sort themselves out into pairs that eventually fuse. Fusion is preceded by several agglutination-induced reactions in the flagella and cell body: (1) flagellar beating changes from a swimming stroke into a twitching movement (Homan et al. 1980); (2) flagellar-tip activation (FTA) occurs, which is the rounding of the flagellar tip as a consequence of the elongation of some outer microtubules (Mesland et al. 1980; Elzenga et al. 1982; Crabbendam et al. 1984); (3) there is a transient increase in agglutinability (Demets et al. 1988); (4) there is transport of agglutinins to the flagellar tip (tipping; Homan et al. 1987); (5) local cell-wall lysis occurs between the bases of the flagella and a plasma papilla protrudes through the anterior cell wall, allowing the gamete to fuse with its partner. The dynamics of these mating responses and their role in mating have been described by Musgrave and van den Ende (1987).

The flagellar components which are responsible for adhesion have been identified and characterized for *C. eugametos* (Musgrave et al. 1981; Homan et al. 1982; Klis et al. 1985; Crabbendam et al. 1987), *C. reinhardtii* (Collin-Osdoby and Adair 1985; Adair et al. 1982) and *C. moewusii* (Samson et al. 1987). In *C. eugametos*, these so-called agglutinins are long stringy, high-molecular-weight glycoproteins extrinsically attached to the flagellar membrane (Musgrave et al. 1981; Homan et al. 1982). Previously, we presented several arguments for a unipolar binding model in which the *mt*− agglutinins bind to the *mt*+ agglutinins, induce the formation of an intracellular signal and consequently trigger mating responses in both gametes (Homan et al. 1988; Kooijman et al. 1989). While the agglutinins and mating responses in all the species are well characterised, the signal transduction mechanism is only recently becoming elucidated. Calcium has often been invoked as a signal in mating *C. reinhardtii* gametes. Kaska et al. (1985) and Bloodgood and Levin (1983) reported the release of immobilized intracellular Ca$^{2+}$ during agglutination and Snell et al. (1982) reported the inhibition of cell fusion by lidocaine. However, intracellular Ca$^{2+}$ concentrations have never been measured and, apart from the isolated report by Claes (1980), no one has been able
artificially to raise the Ca$^{2+}$ level by using calcium ionophores such as A23187 and induce sexual responses (Pasquale and Goodenough 1987; see also this report). In contrast, a role for cyclic AMP (cAMP) in *C. eugametos* gametes was implicated by Pijst et al. (1984), who observed a transient, but dramatic increase in the cAMP level as an immediate consequence of agglutination, and a stimulating effect of dibutyryl-cAMP (db-cAMP) on mating of cells (Pijst 1985). Pasquale and Goodenough (1987) developed this theme further and demonstrated that in *C. reinhardtii*, agglutination also triggers an increase in the cAMP level. They demonstrated that cAMP is involved in sexual signalling by showing that db-cAMP can induce all mating responses in non-mating gametes. This had not been possible in *C. eugametos*. In reassessing the role of cAMP in this species, we studied the effect of wheat-germ agglutinin (WGA) on cAMP levels in gametes because this lectin can evoke all the mating responses, and we tested the effect of exogeneous cAMP on non-mating gametes in the light and in darkness. Only those in darkness responded. In addition, the presence of adenylate cyclase in *C. eugametos* was tested to complement the earlier study on phosphodiesterase and cAMP-dependent protein-kinase activity in this alga (Pijst 1985).

**Material and methods**

**Cell cultures.** The *mt−* strain 5.39.4 and the *mt+* strain 17.17.2 of *Chlamydomonas eugametos* were obtained as described by Schuring et al. (1987) and the cells were cultivated on an agar medium in a 12 h dark/12 h light regimen (Mesland 1976). Gamete suspensions were obtained by flooding two- to four-week-old cultures with a 10 mM Heps [4-(2-hydroxyethyl]-l-piperazineethanesulfonic acid)] buffer, pH 7.6. Non-illuminated cells were obtained by placing the cells in darkness immediately after flooding. Illumination of gametes occurred at a photon fluence rate of 34 lx mol−1 s−1 using white light from fluorescent lamps. The adenylate-cyclase activity was assessed in the *mt+* strain 17.17.2. For the other experiments, the *mt−* strain 5.39.4 was used.

**Reagents.** Phorbol myristate acetate, db-cAMP and db-cGMP were obtained from Sigma, St. Louis, USA. Caffeine was purchased from BDH chemicals, Poole, UK. Calcium ionophore A23187 was purchased from Boehringer, Mannheim, FRG and WGA was obtained from Serva, Heidelberg, FRG.

**Mating reactions.** Twitching was assessed by microscopical observation. Flagellar-tip activation (FTA) was observed and quantitated as described previously (Kooijman et al. 1986).

The presence and localization of active agglutinins was assessed with the aid of the monoclonal antibody Mab 66.3 using an indirect immunofluorescence test (Kooijman et al. 1989). Monoclonal antibody Mab 66.3 binds to the binding site of the *mt−* agglutinin which is exposed on the active but not on the inactive form of the molecule (Homan et al. 1988; Kooijman et al. 1988).

Cells were tested for local cell-wall lysis by enclosing a suspension of gametes on a microscope slide under a coverslip. After 5–10 min, the preparation had dried out to the extent that the cell contents were squeezed out, resulting in the extrusion of a "balloon" between the bases of the flagella (F. Schuring, University of Amsterdam, The Netherlands, personal communication).

Mating-structure activation was observed by indirect immunofluorescence assay using WGA at a concentration of 500 µg ml−1 or Mab 66.3. Wheat-germ agglutinin and Mab 66.3 bind to the exposed papillary membrane and were observed as a fluorescent point between the flagellar bases.

To determine the effect of db-cAMP on the flagellar agglutinability, control cells and db-cAMP-treated cells were fixed in 1.25% glutaraldehyde for 30 min. Subsequently, the fixative was removed by three washing steps in distilled water. The agglutinability of the fixed cells was quantified by determining the highest dilution of the suspension that still exhibited agglutination activity when mixed with five gametes of the opposite mating type.

The fusion competence of the *mt−* cells was determined by mixing them with a tenfold excess of *mt+* cells. After 60 min, the vis-à-vis pairs were fixed in 1.25% glutaraldehyde and the percentage of fusion-competent *mt−* cells was determined by the equation: No. vis-à-vis pairs × 100% / No. *mt−* cells.

**Determination of cAMP.** For cAMP measurements, 100 µl aliquots (containing 1·10⁶–2·10⁶ cells) were extracted in 3 M perchloric acid as described by Pijst et al. (1984). The amount of cAMP was determined using a competition radioimmunoassay based on the method of Steiner et al. (1972). For this assay, a cAMP-specific antiserum together with [125I]cAMP tyrosine methyl ester as a competing agent (Amersham International, Amersham, UK), were used. Acetylation of cAMP, using acetic anhydride and triethylamine, resulted in a sensitivity of 2 fmol cAMP per tube. By incubating the samples with phosphodiesterase, it was shown that the measured values represented cAMP.

**Localization of adenylate-cyclase activity.** The flagella were amputated by pH-shock (Whitman et al. 1972) and separated from the cell bodies as described before (Kooijman et al. 1986). The flagellar membranes were fragmented by sonication and the cell bodies were homogenized by vortexing them with glass beads (0.5 mm diameter) as described by Pijst et al. (1984). Whole-cell homogenates, membrane fractions and cytoplasm fractions were obtained as described by Pijst et al. (1984). Adenylate-cyclase activities were determined according to Janssens et al. (1987).

**Results**

**Responses of cAMP induced by WGA.** The tetravalent lectin WGA can induce all the mating responses in *C. eugametos* gametes (Kooijman et al. 1989). That cAMP

![Fig. 1. Agglutination- and WGA-induced changes in the cAMP levels in *Chlamydomonas* gametes incubated in the light. Cyclic-AMP concentrations were determined after mixing *mt−* and *mt+* gametes (open symbols) or during the incubation of *mt−* gametes in 500 µg ml⁻¹ WGA (closed symbols). The cAMP levels measured during agglutination are the mean levels in the *mt−* and the *mt+* cells.](media.png)