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Protistology

Characterization of phototropin-controlled signaling components that regulate chemotaxis towards ammonium in *Chlamydomonas*

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Summary

Absorption of blue light by phototropin results in the activation of signaling pathways that control the switch-off of chemotaxis during gametogenesis, the conversion of pregametes to gametes, and the maintenance of mating competence in gametes. Pregametes were treated with pharmacological compounds to test their effect on the light-induced switching-off of chemotaxis. The data obtained suggest that a protein kinase C-like component acts as negative regulator early in the signaling pathway while a tyrosine protein kinase and a protein kinase A are needed to transduce the signal downstream from this protein kinase C-like component. The defect of a strain with reduced phototropin levels that, upon irradiation, showed attenuated inactivation of chemotaxis could be corrected by the application of protein kinase C inhibitors or protein kinase A activators, supporting the hypothesis that these protein kinases are components of a phototropin-controlled signaling pathway. A mutant (lrg6) that exhibits light-independent gamete formation was shown also to switch off chemotaxis in the dark. Treatment of this mutant with inhibitors of either tyrosine protein kinase or protein kinase A prevented the switch-off of chemotaxis in the dark, suggesting that the LRG6 gene product acts upstream from both protein kinases. A comparison of the components that make up the three phototropin-controlled signaling pathways operating during sexual differentiation revealed that the chemotaxis-specific route appears to employ moduls from both the pathway that controls gamete formation and the one controlling maintenance of mating ability.

Key words: cAMP, chemotaxis, *Chlamydomonas*, phototropin, protein kinases

Introduction

The biflagellate green alga *Chlamydomonas reinhardtii* uses a broad spectrum of movement behaviors to find positions in the soil, its natural habitat, for optimal growth and reproduction conditions (Nagel et al., 2002; Sineshchekov et al., 2002). Chemotaxis to ammonium enables vegetative cells to orient themselves

towards the preferred nitrogen source. Ammonium also plays an important role in sexual differentiation since lack of an utilizable nitrogen source in *C. reinhardtii* (usually ammonium) is the signal that initiates gamete formation (reviewed in Beck and Haring, 1996).

In gametogenesis, haploid vegetative cells are converted into mating-competent gametes. Gametic differentiation has been shown to be associated with changes not only in cells' biochemistry and subcellular morphology (reviewed in Beck and Haring, 1996) but also in chemotactic behavior of the cells (Ermilova et al., 2003). Unlike vegetative cells and pregametes (generated by incubation of vegetative cells without a nitrogen source in the dark), gametes lack chemotaxis towards ammonium. Loss of chemotaxis towards ammonium by gametes, which is known to play a key role in the repression of their sexual differentiation, may be viewed as one of the cellular adaptations to changing environmental conditions.

Like the development of mating competent gametes, the loss of chemotaxis requires the action of two environmental signals: lack of a nitrogen source and blue light (Treier et al., 1989; Ermilova et al., 2003). Phototropin is the photoreceptor that perceives the light which induces loss of chemotaxis during gametogenesis (Ermilova et al., 2004). Phototropin in *C. reinhardtii* also controls progression of the sexual life cycle. Strains with severely reduced levels of phototropin are impaired in gamete formation, the maintenance of their mating ability, and the germination of zygotes (Huang and Beck, 2003). The downstream signaling pathway that mediates the blue-light-induced conversion of pregametes to gametes has been characterized in some detail at the genetic and biochemical level (Beck and Haring, 1996; Pan et al., 1996; Dame et al., 2002). Three mutants (lrg), which exhibit light independent gamete formation (Gloeckner and Beck, 1995), also showed a loss of chemotaxis towards ammonium in the dark, suggesting that the participating signaling pathways employ shared gene products (Ermilova et al., 2003).

Analysis of the signaling pathway by which light controls the conversion of pregametes to gametes using pharmacological compounds suggests the participation of protein kinases and cAMP in this signaling cascade. Thus, a protein kinase C (PKC)-like activity was shown to operate against signal flux while inhibition of a protein tyrosine kinase (PTK) inhibited signal flux. The intracellular accumulation of cAMP, either by feeding or by the use of phosphodiesterase inhibitors, inhibited the light-dependent conversion of pregametes to gametes suggesting a role of this cyclic nucleotide as a negative regulator (Pan et al., 1996, 1997).

Here we employed pharmacological compounds known to target defined signaling steps. These studies revealed individual elements of the signal transduction pathway involved in the blue-light-controlled change in chemotaxis mode of *C. reinhardtii* during gamete formation: three protein kinases, one operating against signal flux and two that promote signal transduction. The analysis of the expression of genes that served as markers for gametogenesis provided information on shared and divergent elements of the signaling pathway(s) involved in the switching-off of chemotaxis towards ammonium and in the formation of sexually competent cells.

Material and Methods

Strains and culture conditions

Chlamydomonas reinhardtii wild-type strain CC-124 (*mt*$^-$), obtained from the *Chlamydomonas* Culture Collection at Duke University, USA, was used as the tester strain. Another wild-type strain used was CC-620 (*mt*$^+$), obtained from S. Purton, University College London, GB. Strains RNAi20 (*mt*$^-$) with reduced levels of phototropin (Huang and Beck, 2003) and *lrg6* (*mt*$^-$), a mutant showing light independent gamete formation (Dame et al., 2002), have been described previously. Cells were grown at 22° C under a 12-h light/12-h dark regime in Tris acetate phosphate (TAP) medium (Gorman and Levin, 1965) or in acetate-free TAP (TMP) medium.

Reagents

All pharmacological compounds were prepared as stocks and stored at -70° C in the dark. Papaverine and cycloheximide were dissolved in water. Other compounds were dissolved in DMSO when an organic solvent was required, or in TAP-N. Papaverine, 3-isobutyl-1-methylxanthine, chelerythrine, db-cAMP and cycloheximide were purchased from Sigma. Genistein and staurosporine were obtained from Calbiochem-Novabiochem (Bad Soden, Germany). SC-9 was obtained from Biomol (Dianova, Hamburg, Germany). The components were tested at the concentrations that previously have been shown to be effective in mammalian or plant cell systems (Table 1) and affected pregamete-to-gamete conversion in *Chlamydomonas reinhardtii* (Pan et al., 1996). All reagents used did not influence the cellular swimming speed (data not shown).

Generation of pregametes and gametes

For the generation of pregametes, liquid cultures of synchronously growing cells at the beginning of the light period were washed with nitrogen-free medium (TAP-N or TMP-N), resuspended in the same medium at a density of $0.1\text{-}1.0\times10^6$ cells/ml and incubated in

Table 1. Pharmacological compounds that affect light control of chemotaxis

Compound	Mode of action	Effective concentration	Reference
Papaverine	Phosphodiesterase inhibitor	50 μM	Pasquale and Goodenough, 1987
3-isobutyl-1-methylxanthine	Phosphodiesterase inhibitor	50 μM	Pan et al., 1996
db-cAMP	PKA activator	15 mM	Lando et al., 1990.
Genistein	PTK inhibitor	200 μM	Bowler et al., 1994. Pan et al., 1996
Staurosporine	PKC inhibitor, PKA inhibitor	20 nM 50 nM	Zhang and Snell., 1994; Meggio et al., 1995; Pan et al., 1996
Chelerythrine	PKC inhibitor	1 μM	Herbert et al., 1990.
SC-9	PKC activator	200 μM	Ito et al., 1986; Pan et al., 1996

the dark for at least 4 h. Gametes were obtained either by incubation of vegetative cells in nitrogen-free medium with continuous illumination (fluence rate 30μmol$m^{-2}s^{-1}$) or from pregametes by exposing them to light. The light source used was a 500W lamp (LOMO, Russia) mounted in a slide projector. The fluence rates were measured using a photometer (LOMO, Russia). For cells incubated in the dark, manipulations were carried out in a dark room illuminated by a weak red light.

Determination of mating competence

The percentage of gametes was assayed by mixing the cells to be tested with a threefold excess of gametes of opposite mating type. Mating was allowed to proceed in the dark for one hour and stopped by adding glutaraldehyde (final concentration 0.5%). The number of biflagellate cells and quadriflagellate cells in the mating mixture was recorded microscopically. The percentage of mating competent gametes was calculated as described by Beck and Acker (1992).

Chemotaxis assay

Chemotactic responses were tested by counting the cells that in darkness swam into rectangular capillaries (260 μm × 450 μm) filled with 3 μl of medium containing NH_4Cl (5 mM). This number was compared to the number of cells entering capillaries filled with ammonium-free medium (Ermilova et al., 1998). Capillaries were closed at one end with Parafilm. The other end was submerged in the cell suspension for 10 min at 22°C.

The chemotaxis index (CI) was calculated using the following equation:

$$CI = \frac{\text{number of cells entering capillaries filled with medium containing } NH_4Cl}{\text{number of cells entering capillaries filled with } NH_4 \text{ - free medium}}$$

Switch-off of chemotaxis was recorded as 1, 0.

RNA isolation and Northern blot hybridization

Total RNA was isolated and processed for blotting and hybridization as described previously (von Gromoff et al., 1989). Probes for gene *a2, mtd1* (Ferris et al., 2001; Ferris et al., 2002), NSG3 (Abe et al., 2004) and *CBLP*, encoding a *Chlamydomonas* Gβ-like polypeptide (von Kampen et al., 1994), were kindly provided by Dr. Ferris, Dr. Matsuda and Dr. Wettern, respectively.

Results

Analysis of the effect of pharmacological compounds on changes in chemotaxis mode during gametogenesis

Treatment of pregametes with two phosphodiesterase inhibitors, papaverine and 3-isobutyl-1-methylxanthine (IBMX), assumed to result in elevated levels of cAMP, switched off chemotaxis towards ammonium in the dark (Fig. 1). Both inhibitors at the concentrations used did not influence cellular motility (data not shown). The light-induced loss of chemotaxis was not affected by these compounds. An increase in cAMP levels as a result of phosphodiesterase inhibition by papaverine and IBMX is known to activate protein kinase A (PKA).

An involvement of cAMP in the control of chemotaxis is supported by the observation that the feeding of db-cAMP caused a switch-off of chemotaxis in pregametes incubated in the dark (Fig. 2). Upon

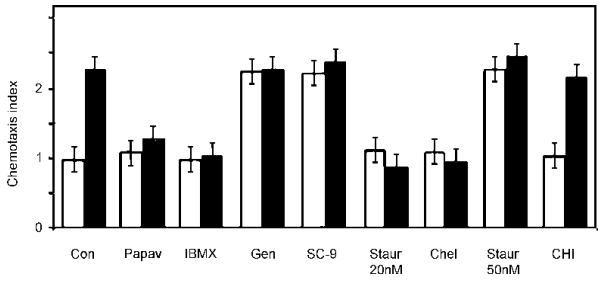

Fig. 1. Effects of various pharmacological compounds on the loss of chemotaxis towards ammonium in pregametes of wild-type strain CC-124 in the light (□) and in the dark (■). Untreated pregametes (Con) served as a control. Compounds were added either to pregametes 10 min (cycloheximide - 1h) prior to start of illumination for 2 h or to pregametes that subsequently were incubated in the dark for an additional 2 h. The concentrations used were 50 μM for papaverine (Papav), 50 μM for IBMX, 200 μM for genistein (Gen), 200 μM for SC-9, 1 μM for chelerythrine (Chel), 20 nM (Staur) and 50 nM (Staur) for staurosporine, 50 μM for cycloheximide (CHI).

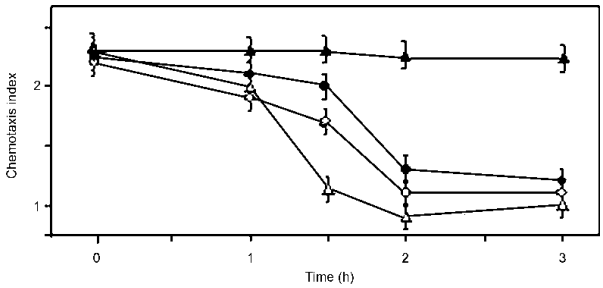

Fig. 2. Influence of db-cAMP on the kinetics of loss of chemotaxis towards ammonium in pregametes of wild-type strain CC-124. Pregametes generated from synchronously growing cells by incubation in TAP-N medium in the dark for 18 h at time 0 were shifted into white light of a fluence rate of 60 μmol m^{-2} s^{-1} (○) or incubated in the dark for an additional 3 h (▲). db-cAMP (15 mM) was added to pregametes 10 min prior to light exposure for 3 h (△), or to pregametes kept in the dark for an additional 3 h (●).

simultaneous exposure of pregametes to light and db-cAMP, a faster switching off of chemotaxis was observed than in cultures only treated with light (Fig. 2). This suggests a promotion of signal flux by activation of a PKA with cAMP.

We next tested various protein kinase inhibitors for their effect on chemotaxis signaling. Addition of genistein, a protein tyrosin kinase (PTK) inhibitor, prevented the light-induced switch-off of chemotaxis in pregametes (Fig.1). In contrast, chelerythrine and low concentrations of staurosporine (20 nM) promoted the switching off of chemotaxis in the dark and had no effect on the loss of chemotaxis induced by light. Staurosporine at low concentrations is a specific inhibitor of PKC, as is chelerythrine (Table 1). Low levels of staurosporine have already been shown to promote pregamete-to-gamete conversion in the dark (Pan et al., 1996). Treatment of pregametes with the naphthalene sulfonamide SC-9, a PKC activator (Ito et al., 1986), specifically inhibited loss of chemotaxis in the light. The combined data suggest that a PKC-like component may be involved in attenuating signaling that results in the loss of chemotaxis.

Staurosporine at higher concentrations (50 nM) blocked the light-induced switch-off of chemotaxis (Fig. 1). It is known that staurosporine may act also as inhibitor of PKA (Meggio et al., 1995). These results suggest that a third protein kinase activity, possibly a PKA which is sensitive to 50 nM staurosporine, is a component of the signal transduction chain by which light controls chemotaxis.

To test whether this light signaling chain is dependent on protein *de novo* synthesis, cycloheximide, an inhibitor of cytoplasmic protein synthesis, was added to pregametes one hour before light exposure. It was noticed that this inhibitor did not block the switch-off of chemotaxis by light (Fig. 1). Thus, all signaling components required for the control of chemotaxis by light appear to be present in pregametes.

Combined treatment of pregametes with activating and inhibiting compounds

In order to gain information on the sequence of the protein kinases in this signaling pathway, we employed a combined application of the activators and inhibitors defined above. Since chelerythrine and staurosporine (20 nM) activated loss of chemotaxis in the dark, we used these compounds in combination with genistein that prevented loss of chemotaxis in the light (Fig. 1). Genistein completely blocked the activation seen with PKC inhibitors chelerythrine and staurosporine (20 nM) (Fig. 3). This implies that the target of genistein in the signaling pathway is located downstream from the PKC. Genistein, however, did not prevent the switch-off of chemotaxis promoted by addition of papaverine, IBMX or db-cAMP in pregametes in the dark. This indicates that a cAMP-targeted PKA operates downstream from the PTK inactivated by genistein. The effects were registered only when the assays were performed within 6 h after removal of the nitrogen source from the medium. No effect of the inhibitors on chemotaxis in vegetative cells was observed. The effects observed in pregametes thus appear not to be caused by interference of these

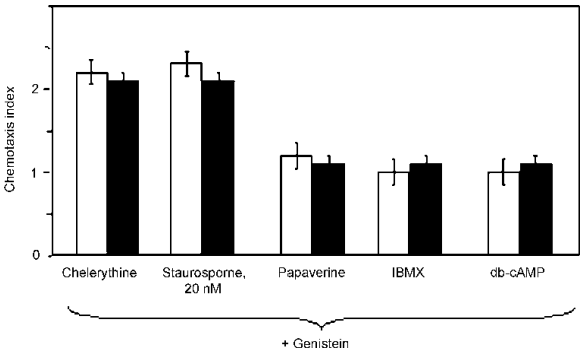

Fig. 3. Treatment of pregametes in the light (□) and in the dark (■) with combinations of pharmacological compounds that either activate or inhibit loss of chemotaxis in pregametes. Compounds were added either to pregametes 10 min prior to start of illumination for 2 h or to pregametes that were incubated in the dark for additional 2 h. The concentrations used were 200 μM for genistein, 1 μM for chelerythrine, 50 μM for papaverine, 50 μM for IBMX, 20 nM for staurosporine.

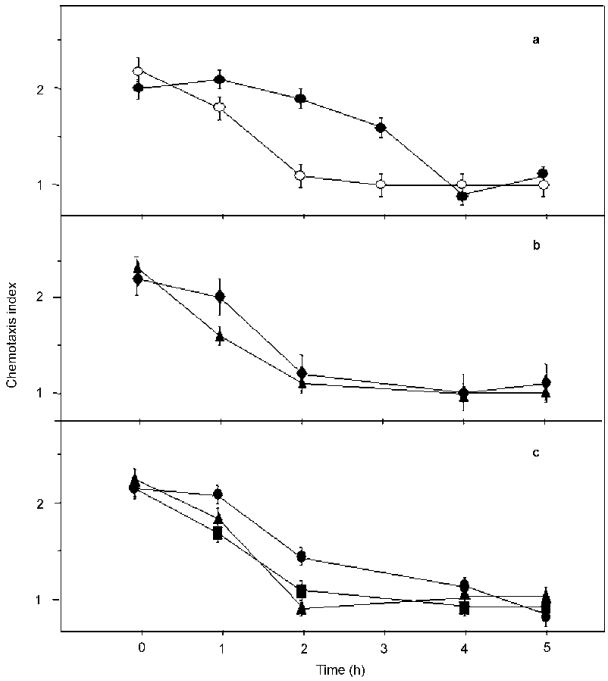

Fig. 4. Effects of various pharmacological compounds on the light-induced loss of chemotaxis towards ammonium in pregametes of phototropin-reduced strain RNAi20. **a** - Non-treated cells. Pregametes of wild type CC-124 (○) and RNAi20 (●) generated from synchronously growing cells by incubation in TAP-N medium in the dark for 18 h, at time 0 were shifted into white light of a fluence rate of 60 μmol $m^{-2} s^{-1}$. **b** - Effect of chelerythrine (▲) or staurosporine, 20 nM (♦) on loss of chemotaxis in pregametes of RNAi20 that were shifted into white light at time 0. **c** - Treatment with db-cAMP (■), papaverine (▲) or IBMX (●) on loss of chemotaxis in pregametes of RNAi20 that were shifted into white light at time 0. Compounds were added to pregametes 10 min prior to start of illumination. The concentrations used were the same as those given in the legend of Fig. 3.

compounds with the signaling mechanism of chemotaxis (data not shown).

The combined data suggest that (i) a PKC-like component with a role as negative regulator is involved in the control of chemotaxis, (ii) a PTK transduces the signal downstream from the PKC-like component, (iii) a PKA transduces the signal downstream from the PTK.

Effect of activators that switch off chemotaxis in the dark on the kinetics of loss of chemotaxis in phototropin-reduced strain RNAi20

We have shown previously that pregametes with reduced levels of the blue-light receptor phototropin exhibit an attenuated light-induced inactivation of chemotaxis (Ermilova et al., 2004). To determine whether the pharmacological compounds tested above target the phototropin-controlled signaling pathway, we analyzed chelerythrine, staurosporine (20 nM), papaverine, IBMX and db-cAMP, compounds shown to activate loss of chemotaxis in the dark (Figs 1 and 2), for their ability to compensate for the slowed-down loss of chemotaxis seen in strain RNAi20. Strain RNAi20, having severely reduced phototropin levels (Huang et al., 2002; Huang and Beck, 2003), exhibited a switching-off of chemotaxis (at a fluence rate of 60 μmol $m^{-2} s^{-1}$) with a delay of about 2 h when compared to parental strain CC-124 (Fig. 4a). After addition of staurosporine (20mM) or chelerythrine, the kinetics of loss of chemotaxis in the light observed for strain RNAi20 were faster than those seen without treatment (Fig. 4b). In the presence of papaverine, IBMX or db-cAMP, the RNAi20 strain exhibited kinetics of loss of chemotaxis in the light that were distinctly more rapid than in untreated cultures and similar to those observed for the wild-type strain (Fig. 4c). These results suggest that a PKC and a PKA, targeted by staurosporine (20 mM) and compounds that elevate cAMP levels, respectively, may be components of the same signaling pathway that is involved in the control of the switch-off of chemotaxis via phototropin-perceived blue light.

Response of a blue-light signaling mutant (*lrg6*) to inhibitors

Light independence of pregamete-to-gamete conversion was observed in mutant *lrg6*. The *LRG6* gene product was suggested to be a negative regulator in the signaling pathway that controls gamete formation

(Dame et al., 2002). This mutant also lost chemotaxis to ammonium in the absence of light (Fig. 5a). The same results have previously been observed for mutants *lrg1, lrg2* and *lrg4*, which affect genes different from *LRG6* (Ermilova et al., 2003). Compounds that inhibited the light-induced switch-off of chemotaxis (Fig. 1) were added to *lrg6* mutant cells incubated in the nitrogen-free medium in the dark. Genistein prevented the loss of chemotaxis (Fig. 5a) as did 50 nM staurosporine (Fig. 5b). Since chemotaxis towards ammonium is lost in mutant lrg6 in the dark but not when the two kinase inhibitors were present, the targets of these inhibitors appear to be positioned downstream from the *LRG6* gene product. Also the PKC defined above appears to operate downstream from LRG6, since loss of chemotaxis in the *lrg6* mutant was inhibited by the PKC activator SC-9 (Fig. 5c). We conclude that the LRG6 protein operates upstream from the protein kinases targeted by genistein, 50 mM staurosporine, or SC-9. If the compounds were added in one hour after ammonium depletion, the effects observed were the same as those shown in Fig. 5. However, genistein, staurosporine (50 nM) and SC-9 did not prevent loss of chemotaxis if they were added 2 h (or later) after removal of the nitrogen source (data not shown).

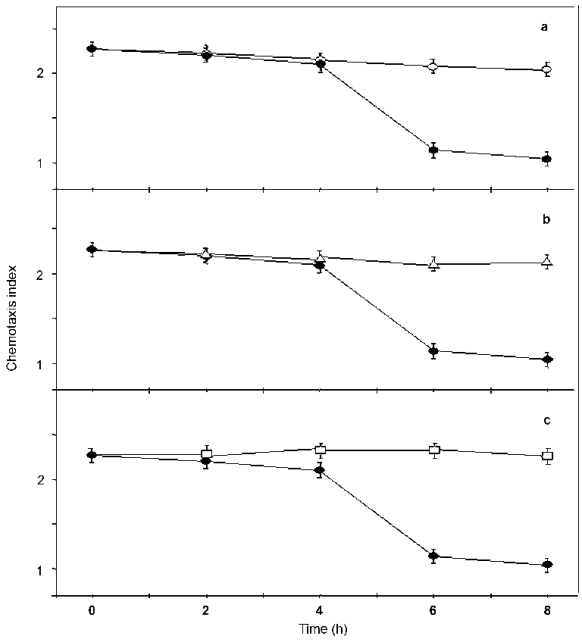

Fig. 5. Kinetics of loss of chemotaxis towards ammonium of the *lrg6* mutant in the dark. Synchronously growing vegetative cells of the *lrg6* strain at time 0 were transferred into the dark and incubated in TAP-N medium. **a** - Cells of the *lrg6* mutant untreated (●) or treated with genistein (○). **b** - Mutant cells treated with 50 nM staurosporine (△). **c** - Culture treated with SC-9 (□).The concentrations used were as given in the legend for Fig. 1.

Effect of staurosporine on the expression of genes upregulated during gametogenesis

Genes that are upregulated during gametogenesis according to their temporal expression patterns may serve as markers for the progression of sexual differentiation (von Gromoff and Beck, 1993; Rodriguez et al., 1999; Abe et al., 2005). Here we analyzed the expression of two gamete-specific genes expressed in both mating types [*a2* and *NSG3*, encoding a cell wall protein and a putative reverse transcriptase, respectively (Ferris et al., 2001; Abe et al., 2004)] and *mtd1*, a gene expressed only in *mt*$^-$ gametes (Ferris et al., 2002). For these genes no accumulation of the respective mRNAs was observed during nitrogen starvation in the dark, i.e., in pregametes (Fig. 6). Light treatment of pregametes for 2 h and, more prominently, for 4 h, resulted in increased levels for *a2, NSG3*, and *mtd1* mRNAs. We tested whether treatment of pregametes with 20 nM staurosporine (that promoted the switch-off of chemotaxis in the dark) and 50 nM staurosporine (that prevented the switch-off of chemotaxis in the light) (Fig. 1) may influence the expression of *a2, NSG3* and *mtd1*. 20 nM staurosporine did not induce the expression of these genes in the dark. However, this same concentration of staurosporine present during irra-diation of pregametes caused elevated mRNA levels of these genes, most prominently those of *a2* (Fig. 6). In the case of 50 nM staurosporine a reduced accu-mulation of *a2* and *NSG3* transcripts was noted after 2 h of irradiation (Fig. 6). Consistent with previous observations, both 20 and 50 nM staurosporine, induced a low level of pregamete-to-gamete conversion in the dark (Fig. 6). A comparison of the patterns of expression of these marker genes for gametogenesis with changes in chemotaxis (Fig. 6) showed a lack of correlation between the responses at the gene level and the protein synthesis-independent loss of chemotaxis.

Discussion

Absorption of blue light by phototropin results in the activation of signaling pathways that control the switch-off of chemotaxis during gametogenesis, the conversion of pregametes to gametes, and the maintenance of mating ability of gametes. Using compounds known to target specific protein kinases, we found that three kinases are likely to play vital roles in the pathway of blue-light-induced loss of chemotaxis in gametes. With the use of staurosporine, two different targets were discovered. One is a PKC-like component that is suggested to operate against signal flux, since its inhibition by 20 nM staurosporine or by chelerythrine induced a light-independent switch-off of chemotaxis

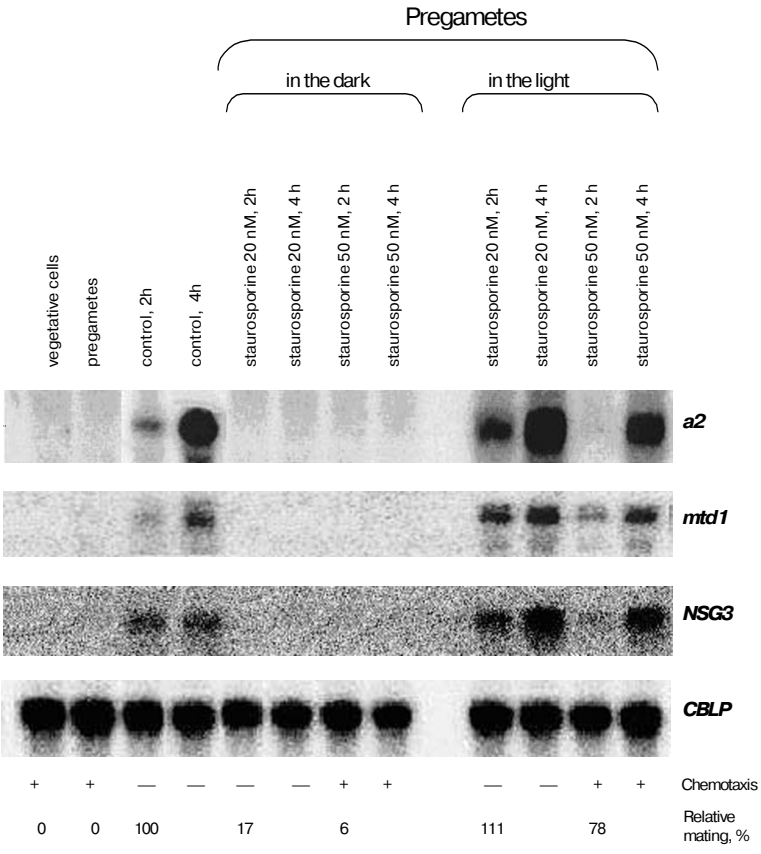

Fig. 6. Effect of staurosporine on the expression of genes *a2, NSG3* and *mtd1*. Pregametes and gametes were generated as described in Material and Methods. Gametes generated from pregametes by light incubation for 2 h or 4 h are labeled "control". Staurosporine was added either to pregametes 10 min prior to light exposure for 2 h and 4 h, or to pregametes kept in the dark for an additional 2 h and 4 h. The RNA gel blots were hybridized with probes specific for genes *a2* and *mtd1*. As a loading control, hybridizations were performed with a probe derived from gene *CBLP* encoding a Gβ-like polypeptide. Maximal mating of pregametes irradiated for 2 h was set as 100%. "+"and "-" refer to cells that do or do not exhibit chemotaxis towards ammonium.

in pregametes (Fig. 1). Such a scenario is supported by the opposite effect of PKC-activator SC-9; this compound blocked the light-dependent switch-off of chemotaxis. Staurosporine at 50 nM inhibited the light-dependent switch-off of chemotaxis (Fig. 1). We consider PKA as a likely target for two reasons. For one staurosporine has been reported to inhibit PKA at concentrations that are about three-fold higher than those required for PKC inhibition (Meggio et al., 1995). For two, a PKA appears to be a likely target of staurosporine since it was independently identified as a component of the signaling pathway: Feeding of cAMP or of phosphodiesterase inhibitors papaverine or IBMX caused a switch-off of chemotaxis in the absence of light (Figs 1 and 2), suggesting that activation of a PKA promoted signal flux.

The combined data have been summarized in a model (Fig. 7). We assume that the signal generated by activation of phototropin feeds into a linear signal chain that switches off chemotaxis towards ammonium. We also assume that a basal flux is going through this signaling chain (Bowler and Chua, 1994), which, however, in the absence of blue-light stimulation is not enough to switch off chemotaxis. In this pathway, a PKC-like component is proposed to act as negative regulator that attenuates transduction of the light signal. Since genistein inhibited the switch-off of chemotaxis induced by 20 nM staurosporine or chelerythrine, a PTK appears to be positioned downstream from the PKC-like activity (Fig. 7). A PKA may be positioned downstream from this PTK since conditions that resulted in a rise of intracellular cAMP levels and thus in an activation of PKA could be shown to promote a switch-off of chemotaxis even when PTK was inhibited by genistein (Fig. 3).

We have previously shown that a reduction in phototropin levels led to a delay in the light-induced switch-off of chemotaxis (Ermilova et al., 2003). By using a strain strongly reduced in phototropin (RNAi 20), we discovered a compensation of this defect upon application of compounds that appeared to activate signal flux: the PKC inhibitors (20 nM staurosporine or chelerythrine) or the PKA activators (db-cAMP or phosphodiesterase inhibitors) (Fig. 4). These protein kinases are thus proposed to be downstream components of the phototropin-activated signaling pathway.

A negative regulator of the blue light signaling pathway that controls gamete formation, protein LRG6, was identified in a mutant screen. Defects in gene *LRG6* caused light independence of pregamete-to-gamete conversion (Dame et al. 2002) and, in addition, showed light-independent switching-off of chemotaxis (Fig. 5), suggesting that the gene product also negatively controls the light signaling pathway for chemotaxis. The inhibiting effect of genistein, SC-9 and 50 nM staurosporine on the loss of chemotaxis in the *lrg6* mutant suggests that the LRG6 protein operates upstream from the protein kinases targeted by the

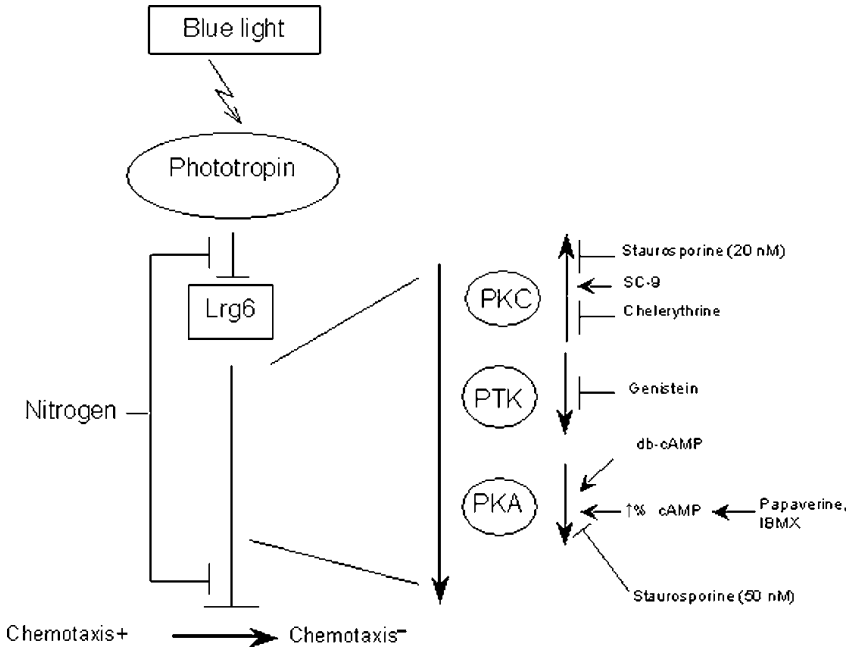

Fig. 7. A model for the signaling pathway by which blue light controls chemotaxis towards ammonium in *C. reinhardtii* during pregamete-to-gamete conversion. Nitrogen (usually ammonium) has been proposed to inhibit blue-light signaling upstream as well as downstream from the LRG6 protein (Dame et al., 2002). Arrows indicate a stimulating effect of pharmacological compounds, T-lines indicate an inhibiting effect.

inhibitors (Fig. 7). This gene is transcribed into two mRNAs postulated to encode two integral membrane proteins with transporter/sensory activity (Dame et al. 2002). One or both of the *LRG6* products is proposed to act as negative regulator(s) in the pathway that controls chemotaxis.

This signaling pathway is similar but not identical to that proposed for the control of blue-light-mediated pregamete-to-gamete conversion: In both pathways a PKC-like activity with attenuating function is followed by a PTK (Fig. 7 and Fig. 6 in Pan et al., 1996). The two pathways though are clearly different, since conditions that lead to elevated intracellular AMP levels promoted loss of chemotaxis in the dark but had an inhibitory effect on the conversion of pregametes to gametes. Possibly, in the two signaling pathways, two PKAs targeted by cAMP operate in different directions with respect to signal flux. While higher cAMP levels promoted the switch-off of chemotaxis and 50 nM staurosporine inhibited this switch-off (Figs. 1 and 2), the opposite was observed during the conversion of pregametes to gametes: Elevated cAMP levels inhibited gamete formation while 50 nM staurosporine was shown to promote it (Fig. 6 and Pan et al. 1996). Another major difference may be assigned to the targets of the signaling pathways: While in the light-controlled conversion of pregametes to gametes the target appears to be the nucleus where gene expression is modulated, the switch-off of gametogenesis may occur in the absence of *de novo* protein synthesis (Fig. 1). This difference is documented by a comparison between the expression patterns of genes up-regulated in response to light during gametogenesis and the modulation of chemotaxis (Fig. 6). Conditions that resulted in a switching off of chemotaxis (i.e., 20 nM staurosporine in the dark) did not activate gene expression. In some respects, the blue light signaling pathway controlling chemotaxis shares features with the one required for the maintenance of sexual competence (Pan et al., 1997). This phototropin-activated pathway (Huang and Beck, 2003), which is independent of protein *de novo* synthesis, shares a PKC-like activity with attenuating function with the pathways that switch off chemotaxis or promote gamete formation. However, lack of inhibition by genistein suggests that a PTK which operates in both the light control of chemotaxis and gamete formation, is absent in this signaling pathway. These data suggest a modular make up of the three signaling pathways, possibly employing identical components. One component shared by all three pathways may be the PKC-like activity. A PTK was shown to be a common component of the pathways that control chemotaxis and gamete formation. Possibly also a PKA is shared by these two pathways; in this case the kinase involved in the control of chemotaxis operates in the direction of signal flux, while that controlling gamete formation is postulated to operate in opposite direction. A modular construction of signaling pathways is well documented in *Saccharomyces cerevisiae*, where multiple components of three different MAP kinase signal transduction cascades are shared (Schwartz and Madhani, 2004). In yeast, these components have been identified at the molecular level. The analysis presented here should allow us to identify some of the key components of the blue light signaling chains that control chemotaxis, gametogenesis and maintenance of mating competence.

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