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Molecular characterization of two light-induced, gamete-specific genes from *Chlamydomonas reinhardtii* that encode hydroxyproline-rich proteins

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Abstract Gametic differentiation in *Chlamydomonas reinhardtii* is a two-step process, which is controlled by the sequential action of the two extrinsic signals, nitrogen starvation and blue light. The gamete-specific genes *GAS28* and *GAS29* are expressed in the late phase of gametogenesis. Their light-induced expression is restricted to cells that have completed the first, nitrogen starvation-activated, phase of differentiation. A comparison of the two genes revealed striking similarities as well as differences. Their most prominent shared feature is an extended sequence homology of over 90% in their 5′-untranslated regions, suggesting a role in translational regulation. *GAS28* and *GAS29* both encode hydroxyproline-rich proteins (HRGPs) of very similar sizes that exhibit typical features of volvocalean cell wall constituents. *GAS28* shows a high degree of homology with the *Volvox* pherophorin gene family, suggesting a relationship between these genes.

Key words Cell wall proteins · *Chlamydomonas reinhardtii* · Gamete-specific genes · Gene expression patterns · Hydroxyproline-rich glycoproteins

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Introduction

The initial step in the sexual life cycle of the heterothallic unicellular green alga *Chlamydomonas reinhardtii* is the differentiation of vegetative cells into gametes. Gametes of opposite mating types (*mt*+ and *mt*−) may then fuse to form diploid zygotes. These cells synthesize a thick cell wall and can survive adverse environmental conditions such as dessication and low temperatures (Harris 1989; Beck and Haring 1996). On germination meiosis occurs, resulting in formation of four haploid products (Levine and Ebersold 1960).

Gamete formation in *C. reinhardtii* is controlled by two environmental cues. It is initiated by the removal of a utilizable nitrogen source (usually ammonium) from the culture medium (Sager and Granick 1954; Kates and Jones 1964; Beck and Acker 1992; Matsuda et al. 1992). When incubated in nitrogen-free medium in the dark, cells differentiate into mating-incompetent pregametes. These pregametes can be converted into sexually mature gametes by irradiation with blue light (Treier et al. 1989; Weissig and Beck 1991; Beck and Acker 1992).

Gametic differentiation has been shown to be associated with changes in cell biochemistry and subcellular morphology. Morphologically, the acquisition of two new organelles, a mating structure and a special type of Golgi-derived vesicle are most prominent (Friedman et al. 1968; Martin and Goodenough 1975). At the molecular level, modifications that occur during gamete formation include a reduction in the number of cytoplasmic and organelle ribosomes (Siersma and Chiang 1971; Martin and Goodenough 1975; Martin et al. 1976), the appearance of ribosomes with altered properties (Picard-Bennoun and Bennoun 1985; Bulté and Bennoun 1990), the synthesis of agglutinins (Wiese 1965; Adair 1985; Saito et al. 1985), and the synthesis of gamete autolysin (Waffenschmidt et al. 1989; Kinoshita et al. 1992). The photosynthetic apparatus is also affected during gametogenesis, as shown by the destabilization and degradation of the cytochrome b_6f complex

(Bulté and Wollman 1992). These observations have suggested that gamete formation is based on changes in the pattern of expression of multiple genes (Treier and Beck 1991). In fact, the expression of a gamete-specific set of genes has been documented. Among these are the *GLE* gene, which encodes a gametic lytic enzyme (Kinoshita et al. 1992), the gene for L-amino acid oxidase (Vallon et al. 1993), and the *GAS* genes, which encode proteins of as yet unknown function (von Gromoff and Beck 1993).

One approach to the understanding of gametogenesis at the molecular level relies on the identification and characterization of genes that are expressed during gametogenesis, i.e., genes whose mRNAs are absent or present at very low levels in vegetative cells but accumulate in the course of gametic differentiation. The characterization of such gamete-specific genes serves several purposes: (1) the identification of such genes and of their gene products may provide information on the function of molecules involved in gametogenesis; (2) characterization of their regulatory regions offers a route to the analysis of the regulatory cascade that controls these genes; and (3) they may help to define the positions of mutational blocks in gametogenesis. In fact, the availability of genes that are induced at different stages during the temporal program of gametogenesis has provided molecular tools for determining the approximate stages at which gametogenesis-defective mutants are blocked (Buerkle et al. 1993; von Gromoff and Beck 1993; Gloeckner and Beck 1995). Four genes that are specifically expressed during gametogenesis in *C. reinhardtii* have previously been cloned and their expression patterns analyzed. mRNAs encoded by these gamete-specific genes (*GAS*) are absent or present only at very low levels in vegetative cells and mature zygotes. The mRNAs produced by two of these genes (*GAS3, GAS96*) begin to increase early during gametogenesis, while others are activated during the late phase (von Gromoff and Beck 1993).

Here we report on the isolation and molecular characterization of two related gamete-specific genes. Both genes encode hydroxyproline-rich proteins (HRGPs) that could be constituents of the cell wall of the *C. reinhardtii* zygote. These genes exhibit similar regulatory patterns and show striking homology in their 5′ untranslated regions. The high degree of homology of GAS28 with the *Volvox* pherophorin gene family suggests a functional relationship between these proteins.

Materials and methods

Strains and culture conditions

The *C. reinhardtii* wild-type strain CC-124 (*mt*−), from the Chlamydomonas Genetic Center (Duke University, Durham, N.C.) was used in the gene expression experiments. As a mating partner, strain CF14 (*mt*+) (obtained from a cross between wild-type strains) was used. For genomic DNA gel blots, the wild-type strain 137c (*mt*+), kindly provided by R. Matagne (University of Liège, Belgium), was utilized. The gametogenesis-defective mutant C4 (Bulté and Bennoun 1990), as well as the mutants lrg3 and lrg4, which exhibit light independent gametogenesis (Gloeckner and Beck 1995), have been described previously. Cells were grown photomixotrophically at 23° C in a TRIS acetate phosphate (TAP) medium (Gorman and Levine 1965) on a rotary shaker (120 rpm). Continuous illumination (white light) at a fluence rate of either 40 or 60 μmol/m^2 per s was provided by fluorescent tubes (Osram L36W/25).

Gametogenesis and assay of mating ability

For induction of gametogenesis, vegetative *C. reinhardtii* cultures at a density of approximately 5×10^6 to 1×10^7 cells/ml were concentrated by centrifugation (3000 ×*g* for 5 min). TAP medium was carefully removed and the cells were resuspended in nitrogen-free TAP medium (TAP-N) at a density of about 5×10^6 cells/ml. Subsequently cells were incubated with shaking for 5–17 h. Pregametes were generated by incubating the cells in nitrogen-free medium with shaking in the dark for 14–15 h. Mating and determinations of the percentage of mating were performed as described previously (Treier et al. 1989; Beck and Acker 1992).

Isolation of cDNA clones

The isolation of the *GAS28* cDNA has been described previously (von Gromoff and Beck 1993). For the isolation of *GAS29*, a cDNA library generated from *mt*+ gametes in λZAPII (kindly provided by W. Snell) was screened using probes from the 5′ end (an *Eco*RI-*Sst*I fragment of 745 bp) and 3′ end (a *Nhe*I-*Eco*RI fragment of 650 bp) of the *GAS28* cDNA. The probe from the 5′ end hybridized with clones carrying the *GAS28* gene and also with other clones carrying a new gene, called *GAS29*. The 3′ end probe hybridized only with *GAS28*. The cDNA inserts (*GAS28*, 2.4 kb; *GAS29*, 2.3 kb) were subcloned in pBluescript SK(+) as *Eco*RI-*Xho*I fragments. Plaque lifts and hybridizations were done using nylon membranes (Amersham) according to the protocol provided by the manufacturer.

DNA sequencing and sequence analyses

Subclones for sequencing were generated by cleaving the cDNAs with the restriction enzymes *Rsa*I, *Taq*I and *Sau*3AI and cloning the different fragments in pBluescript SK(+). All clones were sequenced using the ALF semi-automated nonradioactive sequencing system (Pharmacia). DNA sequences were assembled and analyzed using the programs DNAsis, PC-gene or PROSIS. Protein sequences were aligned with the SIM program at Expasy (http://www.expasy.ch/sprot/sim-prot.html). The sequences have been deposited in Genbank under the Accession Nos. AF015883 (*GAS28*) and AF015884 (*GAS29*).

Genomic Southern analysis

Genomic DNA was isolated by the CTAB method (Murray and Thompson 1980). The DNA was subsequently digested with restriction enzymes, then subjected to electrophoresis on 0.7% (w/v) agarose gels, and transferred to Hybond-N membranes (Amersham) by capillary transfer, using 10 × SSC or 25 mM sodium phosphate buffer (pH 6.5). The DNA was cross-linked to the membranes by exposure to UV radiation. The blots were hybridized with one of the following probes: GAS28 3′ end probe (a 687-bp *Nhe*I-*Xho*I fragment from the 3′ end of the GAS28 cDNA); GAS29 3′ end probe (a 670-bp *Sst*II-*Xho*I fragment from the 3′ end of the GAS29 cDNA); and GAS28 5′ end probe (a 745-bp *Eco*RI-*Sst*I fragment from the 5′ end of GAS28). The labeling of probes, prehybridizations and hybridizations were performed as described previously (Wegener and Beck 1991). Washes were done with increasing stringency, culminating in a final wash at 65° C in

0.1 × SSC, 1% SDS for 30 min. Membranes were exposed to X-ray films at −70° C with intensifying screens for 24 h.

Northern analysis

For RNA isolation, cells were harvested by centrifugation for 5 min at 4° C, resuspended in 2× lysis buffer (0.6 M NaCl, 10 mM EDTA, 100 mM TRIS-HCl pH 8, 4% SDS), and then frozen in liquid nitrogen. The lysates were extracted with phenol (Sambrook et al. 1989). The RNA was precipitated with 0.33 vol of 8 M LiCl at 4° C overnight (Barlow et al. 1963). The pellet was suspended in diethylpyrocarbonate-treated water, mixed with 0.1 vol of 3 M sodium acetate (pH 6) and precipitated with 2.5 vol of ethanol at −20° C. Total RNA (10 µg/lane) was fractionated on 1.2% (w/v) agarose/formaldehyde gels, transferred to Hybond-N membranes (Amersham), using 25 mM sodium phosphate buffer (pH 6.5) and baked at 80° C for 2 h. The probes used for hybridization were as follows: the GAS28 probe was a 1.6-kb *Sst*I-*Xho*I fragment from the 3′ end of the GAS28 cDNA, and the GAS29 probe a 2.0-kb *Hin*dIII-*Xho*I fragment of the 3′ end of the GAS29 cDNA. The *CBLP* gene, which encodes a Gβ-like polypeptide (von Kampen et al. 1994), was used as a standard. Labeling of probes, hybridizations, washes and autoradiography were performed as described previously (Wegener and Beck 1991).

Results

Cloning and sequencing of the GAS28 and GAS29 cDNAs

In a previous paper (von Gromoff and Beck 1993) we reported that the gamete-specific gene *GAS28* was present in a single copy in the genome. However, when we used the 5′ end of the GAS28 cDNA as a probe, two hybridizing bands appeared in digests of total *Chlamydomonas* DNA (Fig. 1). In order to determine whether other genes with homology to *GAS28* were expressed, we screened a gamete (*mt*+)-specific cDNA library using probes from the 5′ end and the 3′ end of *GAS28* (an *Eco*RI-*Sst*I fragment of 745 bp and a *Nhe*I-*Eco*RI fragment of 650 bp, respectively). We found several clones that hybridized to the 5′ probe but not to the 3′ probe. These clones harbored a different cDNA that was named GAS29. *GAS28* accounted for about 90% of the cDNA clones detected in the gamete-specific library, and *GAS29* made up the remaining 10%, suggesting that their transcripts are expressed at different levels. The cDNAs of *GAS28* and *GAS29* were cloned in pBluescript SK(+) as *Eco*RI-*Xho*I fragments and their nucleotide sequences were determined. Both cDNAs are similar in length: GAS28 is 2389 bp long and GAS29 2287 bp. DNA sequence analysis revealed ORFs of 1338 bp for *GAS28* and of 1299 bp for *GAS29*.

The untranslated regions in both genes are also similar in size. Thus, *GAS28* has a 368-bp 5′ untranslated region (5′ UTR) and a 677-bp 3′-untranslated region (3′ UTR); the *GAS29* untranslated regions comprise 311 bp at the 5′ end and 653 bp at the 3′ end. In both genes there is a *Chlamydomonas* polyadenylation signal (TGTAA) 17 bp (*GAS28*) and 8 bp (*GAS29*) upstream of the poly(A) tail. The hybridization results with the *GAS28* and *GAS29* 3′ ends as probes confirm that both genes are single copy and are localized on different restriction fragments (Fig. 1).

A unique feature of *GAS28* and *GAS29* is the extremely high homology found between their 5′ UTRs (Fig. 2). In fact, the first 250 bp of the 5′ UTR of *GAS29* is 93% identical to the same region of the GAS28 cDNA. This is the region that is responsible for the cross-hybridization between the two cDNAs. The 5′ region of *GAS28* was used in a BLAST search in all current databases but did not show any significant homology to published sequences. We were unable to detect secondary structure in the 5′ UTR, nor are any significant DNA repeats present. There are no other ATG codons upstream of those indicated. In addition, the sequences surrounding the start codon comply with the consensus sequence for initiation of translation in plants (Cavener and Ray 1991; Kozak 1984).

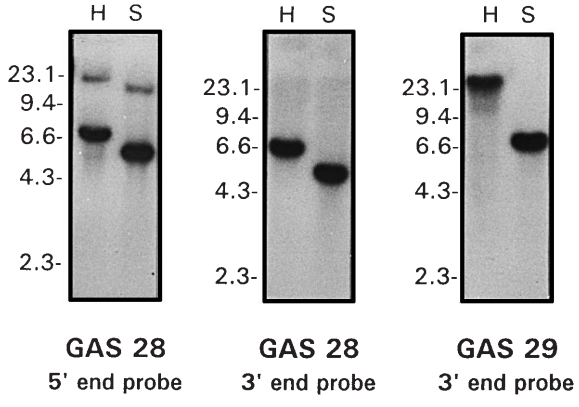

Fig. 1 Southern analysis of total genomic DNA from *C. reinhardtii*. DNA was digested with the enzyme *Hin*dIII (H) or *Sal*I (S), fractionated on a 0.7% agarose gel, blotted and hybridized with the probes indicated, as described in Materials and methods. Sizes of markers (indicated on the left) are given in kb

GAS28 and *GAS29* encode different hydroxyproline-rich glycoproteins

The deduced amino acid sequences of *GAS28* and *GAS29* are shown in Fig. 3. The ORFs encode proteins of nearly identical sizes: 446 amino acids in the case of *GAS28* and 433 amino acids in the case of *GAS29*. The corresponding molecular masses predicted from the sequences are 47.4 kDa and 46.6 kDa for GAS28 and GAS29, respectively. Although the sequences of the predicted gene products are dissimilar, they show some common features. A conserved hydrophobic secretion signal of 29–30 amino acids was identified for both *GAS28* and *GAS29*. Both gene products have proline-rich regions, which might be subject to hydroxylation. Thus, GAS28 protein contains a Ser(Pro)x central domain, 66 amino acid residues long, that is composed nearly exclusively of prolines (59%) and serines (14%). On the other hand, GAS29 exhibits a 37-amino acid

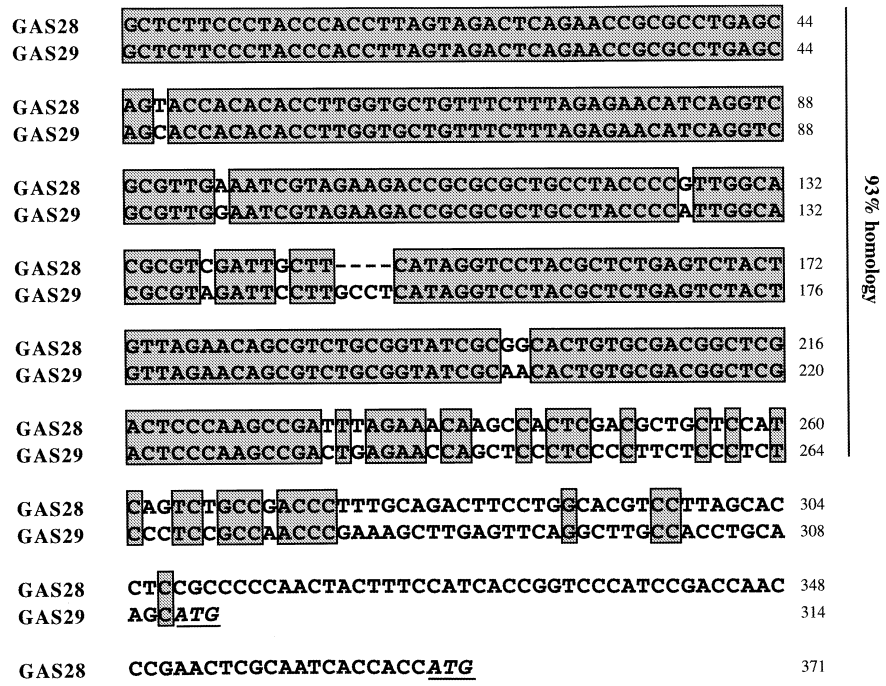

Fig. 2 Homology of the 5′ untranslated regions of *GAS28* and *GAS29*. Matching bases are *boxed and shaded*. The presumptive translational start codons are *underlined*

proline-rich domain close to its N-terminus; in this case prolines represent 73% of the residues and serines 11%. In the GAS29 protein there is an uninterrupted stretch of 17 prolines, whereas in the GAS28 protein the proline clusters are shorter and interrupted by serines. Both proteins contain two potential sites for N-linked glycosylation (Asn X Ser/Thr). Thus, these proteins have the characteristics of hydroxyproline-rich glycoproteins. The rest of both proteins is strikingly rich in cysteine residues. Thus, in the N-terminal domain of GAS28 there are 13 cysteines and 14 more are found in the C-terminal domain. In contrast, there are no cysteines in the short N-terminal domain of GAS29, but 16 in its C-terminal region. In the GAS28 protein there are two repetitive peptide sequences (I and II, Fig. 3) that contain several cysteines. These cysteines are evenly spaced throughout domain II, suggesting that disulfide bridges could play a role in the folding of this protein. The repetitive motifs are separated by the proline-rich region, giving the GAS28 protein a modular structure with two similar globular domains.

Expression of *GAS28* and *GAS29* during gametogenesis

Both *GAS28* and *GAS29* encode mRNAs of about 2.4 kb. Their expression levels are very low in vegetative cells but are strongly enhanced in the late phase of gametogenesis, i.e., 7–11 h after removal of the nitrogen source from the medium (Fig. 4). High mRNA levels are maintained during subsequent incubation of the gametes. In the case of *GAS28*, a small increase in RNA levels is seen at early time points but this has not been observed in other experiments. Though the kinetics of RNA accumulation are similar for *GAS28* and *GAS29*, the profiles differ in detail. Thus, *GAS28* mRNA levels start to rise rapidly prior to 7 h after induction of gametogenesis, reaching maximal levels around 11 h. In contrast, *GAS29* mRNA increases more slowly and significant levels are observed only 11 h after the start of gametogenesis, reaching a peak value around 14 h. As a loading control, the constitutively expressed gene *CBLP*, encoding a Gβ-like polypeptide (von Kampen et al. 1994), was used. The expression of both genes clearly is induced during gametogenesis, but is not related to the occurrence of mature gametes (Fig. 4). A significant number of gametes (38%) is already observed after 5 h, when the levels of *GAS28* and *GAS29* mRNAs are still low.

Because gametogenesis proceeds in two steps, the final step being dependent on light, we tested whether these genes were light inducible. In pregametes, generated by incubation without a nitrogen source in the dark for 14 h, *GAS28* and *GAS29* mRNA levels corresponded to those in vegetative cells. These pregametes were illuminated for 5 h and the appearance of gametes in the culture was monitored, together with the expression of *GAS28* and *GAS29* (Fig. 5). Again, no correlation between *GAS28*/*GAS29* mRNA accumulation patterns and gamete maturation was observed, although the expression of these genes can be induced by light. The first gametes were detectable within 1 h after the start of illumination, but the increase in *GAS28* and *GAS29* mRNA levels occurred only after 5 h. During the conversion of pregametes to gametes the kinetics of transcript accumulation were similar for both genes.

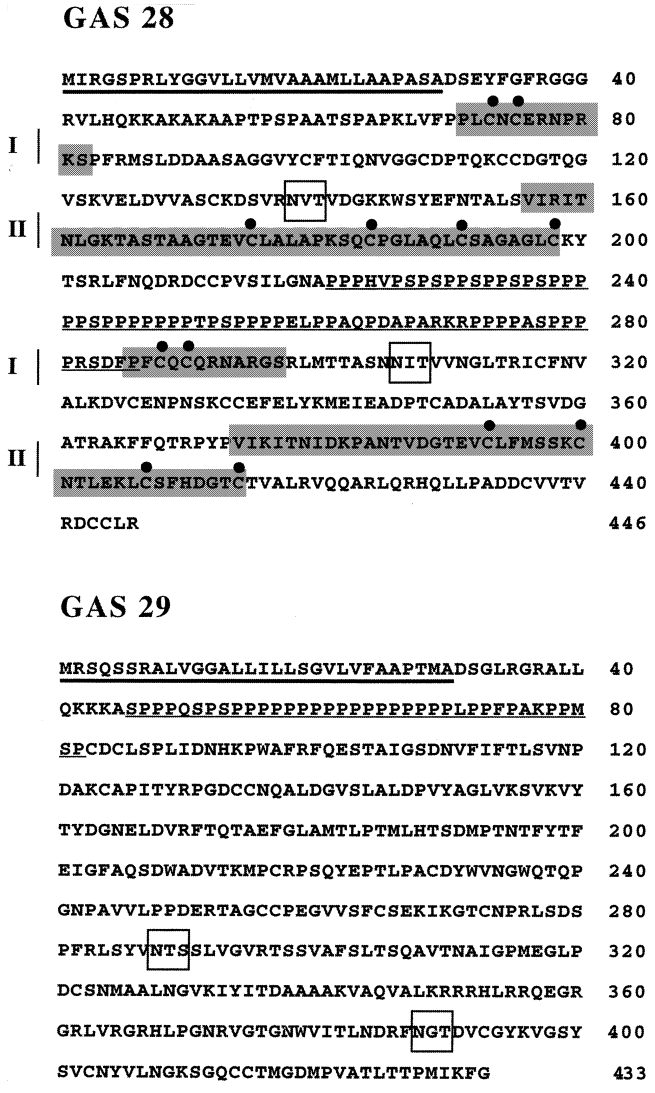

Fig. 3 Deduced amino acid sequences of the products of *GAS28* and *GAS29*. The (SerPro)-rich domains are *underlined*, conserved secretion signals are *underlined in bold*, and potential sites for N-linked glycosylation are *boxed*. The repetitive peptide sequences I and II in the GAS28 protein are *boxed and shaded* and cysteine residues are marked by *dots*

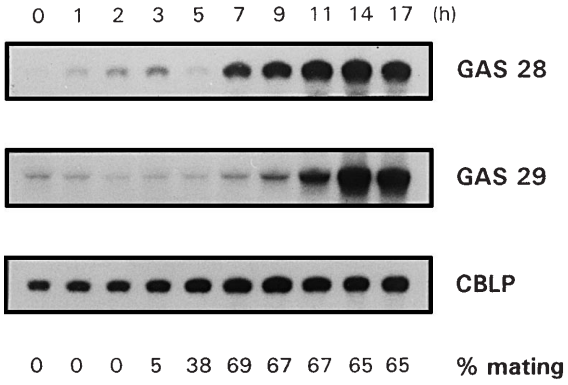

Fig. 4 Kinetics of *GAS28* and *GAS29* mRNA accumulation during gametogenesis in wild-type *C. reinhardtii* cells. The nitrogen source was removed at time 0 and cells were incubated with continuous illumination at a fluence rate of 60 μmol/m^2 per s. Samples for RNA isolation were removed at the times indicated (h). Northern blot hybridizations were performed as described in Materials and methods. Hybridization with the *CBLP* probe was used as a loading control. The percentage of mating at each time point is given *below* the autoradiograms

Expression of *GAS28* and *GAS29* in gametogenesis mutants

There are several *Chlamydomonas* mutants that are able to form gametes in the dark (Gloeckner and Beck 1995). We have shown before that some of these mutations influence the expression of gametogenesis-related genes (Buerkle et al. 1993; Gloeckner and Beck 1995). We tested the expression of *GAS28* and *GAS29* in two different mutants that exhibit light-independent gametogenesis (Fig. 6). The lrg3 mutant can form gametes completely independently of light, while in the lrg4 mutant in the dark, only 25% of the maximal number of gametes develop (Gloeckner and Beck 1995). Although the expression of *GAS28* and *GAS29* is dependent on light in the wild-type strain, the transcript levels of these genes in the lrg3 mutant are high under both light and dark conditions (Fig. 6). In the lrg4 mutant also, the expression of both genes is independent of light. However, in the lrg4 mutant, the *GAS28* gene appears to be repressed again after 11 h in the dark, while *GAS29* mRNA levels remain high. In both lrg mutants elevated levels of *GAS28* and *GAS29* mRNA were observed only after induction of gametogenesis by nitrogen starvation (results not shown).

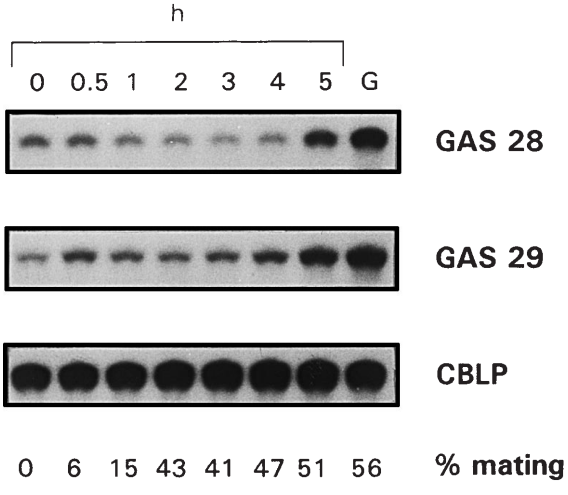

Fig. 5 Changes in the amounts of *GAS28* and *GAS29* mRNAs during the pregamete-to-gamete conversion. Pregametes of wild-type cells were generated by starvation for nitrogen in the dark for 14 h. Starting at time 0, pregametes were irradiated with a fluence rate of 40 μmol/m^2 per s, and samples for RNA isolation were removed at the times indicated (h). As a control, RNA from gametes (G) was included (cells continuously illuminated at the same fluence rate for 14 h after removal of the nitrogen source). As a loading control a probe for the gene *CBLP* was used. The percentage mating is given *below* the autoradiograms

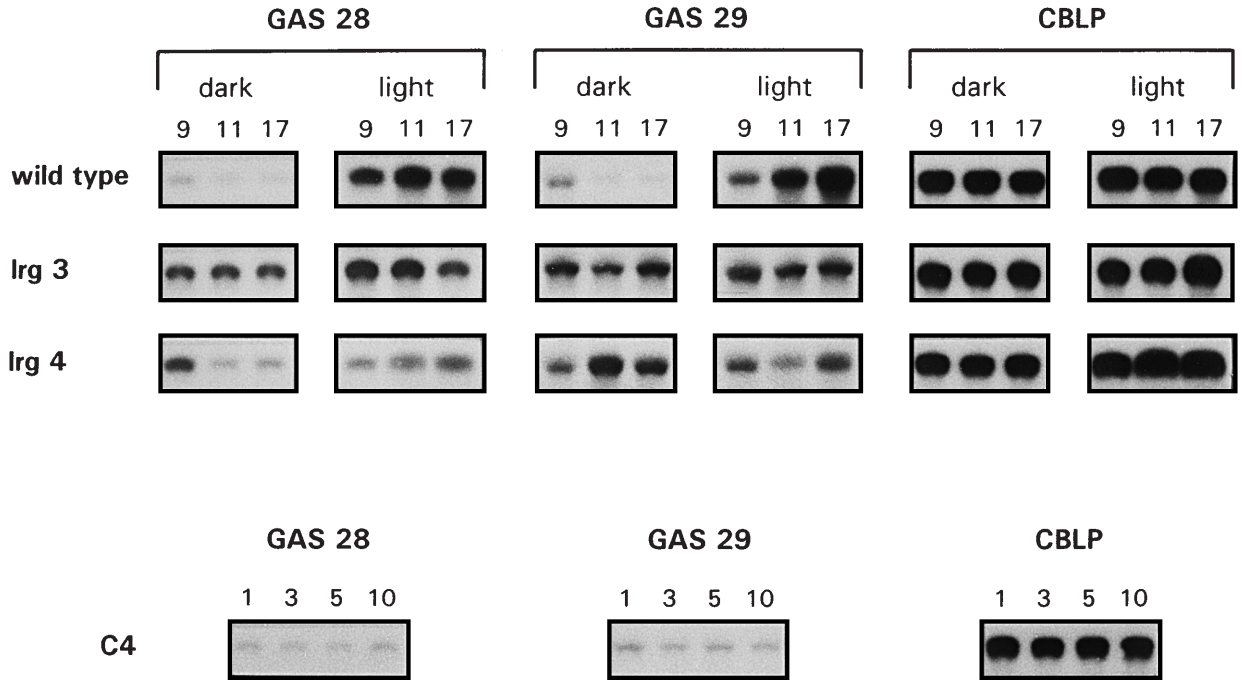

Fig. 6 Accumulation of *GAS28* and *GAS29* mRNAs during gametogenesis in a wild-type strain and in the mutants lrg3, lrg4, and C4. The nitrogen source was removed at time 0 and cells were incubated either in the light (fluence rate: 60 μmol m^2 per s) or in the dark. In the case of mutant C4, cells were incubated only in the light. Samples for RNA isolation were removed at the times indicated (h). The filters containing mutant and wild type RNAs were auroradiographed for the same lengths of time. Gene *CBLP* served as a loading control

In the *Chlamydomonas* mutant C4 (Bulté and Bennoun 1990), which is incapable of forming gametes, the expression of both *GAS* genes during nitrogen deprivation in the light remains at the levels observed in vegetative cells. The expression of the *CBLP* gene is not affected by this mutation.

Discussion

We describe here the characterization of two single-copy genes that are specifically expressed during gametic differentiation in *C. reinhardtii*. The two genes, *GAS28* and *GAS29*, exhibit several features that suggest that they are closely related. The structures of both genes are rather similar at the cDNA level, when the sizes of the 5′ and 3′ UTRs as well as the coding regions are compared. Thus, the predicted gene products comprise 446 and 433 amino acids for *GAS28* and *GAS29*, respectively. The most striking similarity between these two genes, however, concerns their 5′ UTRs where 93% of the bases are identical over a length of 250 bp (Fig. 2). The expression patterns of *GAS28* and *GAS29* are also rather similar (Fig. 4). Both genes are only weakly expressed in vegetative cells and during the first few hours after initiation of gametogenesis. Their mRNA levels only rise in the late phase when mating-competent gametes are already present. These kinetics suggest that neither of the gene products is needed for the formation of sexually competent gametes. The dependence of both genes upon light as an activator (Fig. 5) is compatible with their late expression in gametogenesis, i.e., during the light-controlled phase (Beck and Acker 1992; Beck and Haring 1996) . This similarity in regulation suggests that both genes are subject to control by similar or even identical regulatory networks. However, in contrast to all these similarities, which would suggest a close evolutionary relationship, their nucleotide sequences (with the exception of the highly conserved 5′ UTRs) as well as the amino acid sequences of the predicted products, are dissimilar. This lack of homology suggests that the two genes have different evolutionary origins. To account for the similarities in expression pattern, as well as for the homology in the 5′ UTRs, we suggest that in the course of evolution both genes may have acquired a common sequence upstream from the translation initiation site. Another instance of this has recently been found in an HRGP gene, which shares the complete 5′ region with an unrelated gene in the mating type locus (P. Ferris, unpublished results).

Despite the general dissimilarity in the amino acid sequences, *GAS28* and *GAS29* do show some common features at the protein level. Both genes encode hydroxyproline-rich proteins with conserved secretion signals (Fig. 3). This, and the presence of potential sites for N-linked glycosylation, suggests that both gene products are components of the cell wall (Woessner and Goodenough 1992). Other genes that initiate transcription within minutes of cell fusion have already been described (Ferris and Goodenough 1987). Of these, one has been shown to encode a protein in the zygote cell wall (Woessner and Goodenough 1989). We speculate

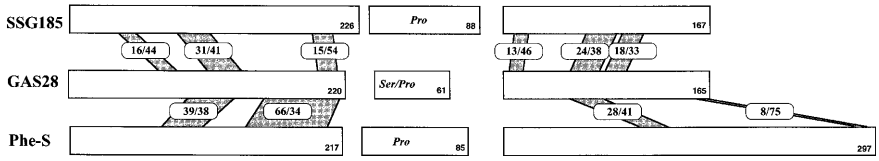

Fig. 7 Homology of GAS28 protein to two members of the *Volvox* pherophorin gene family, Phe-S and SSG185. N- and C-terminal parts of the proteins were aligned separately. Homology is expressed as percent sequence identity. The N-terminal and C-terminal domains are separated by serine/proline-rich (*Ser/Pro*) or proline-rich (*Pro*) regions. The lengths of the protein domains (in amino acids) are indicated

that the GAS28 and GAS29 proteins are constituents of the cell wall formed by young zygotes and that the *GAS28* and *GAS29* mRNAs accumulated in the late phase of gametogenesis may be stored until activated for translation through a signal elicited by cell fusion.

Database searches with the GAS28 protein sequence revealed that it has a significant homology with the *Volvox* extracellular matrix proteins SSG185 (Ertl et al. 1989) and Pherophorin-S (Godl et al. 1997). The GAS28 protein appears to be structurally similar to these *Volvox* proteins, as it also consists of two globular domains separated by a proline-rich spacer (Fig. 7). Alignment of the N-terminal domains of these three proteins shows that there are highly homologous motifs in these three HRGPs. These conserved regions are rich in cysteines. The C-terminal part of Phe-S differs both in size and amino acid sequence from those of GAS28 and SSG185, suggesting this is a structurally different domain. Both SSG185 and Phe-S are proteins of the extracellular matrix of *Volvox*, localized in the cellular zone and the deep zone of the spheroids, respectively. They are not components of the somatic cell wall. This would parallel our suggestion that *GAS28* encodes a protein that is not present in the gametic cell wall, but will become part of the zygote cell wall that is formed after fusion.

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