Carbon dioxide and light regulation of promoters controlling the expression of mitochondrial carbonic anhydrase in *Chlamydomonas reinhardtii*

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Nuclear genes coding for carbonic anhydrase, a major mitochondrial constituent in *Chlamydomonas reinhardtii* grown under limited CO\(_2\), were characterized. Two genes, *ca1* and *ca2*, were found within 7 kb of genomic DNA, organized ‘head to head’ in a large inverted repeat. The DNA sequences for the two genes were very similar, even in the promoter regions and in introns, indicating that the repeat is a result of a recent duplication. To study gene regulation, elements from the upstream region of *ca1* were fused to the arylsulphatase reporter gene. After transformation, the expression of arylsulphatase was regulated similarly to the endogenous *ca1/ca2* genes, even when the promoter was trimmed down to 194 nt. Expression could not be detected when 5\% CO\(_2\) was bubbled into the growth medium, but was induced within hours after transfer to air. The *ca1* promoter was not induced in low light, but at intermediate light levels its activity was dependent on the irradiance. O\(_2\) concentration had no effect on the promoter activity, indicating that photorespiratory metabolites are not triggering the response. The availability of cells transformed with a CO\(_2\)-regulated reporter gene should facilitate further studies on the metabolic adaptations that occur in some green algae in response to the external CO\(_2\) level.

**INTRODUCTION**

The eukaryotic green alga *Chlamydomonas reinhardtii* is one of the microalgal species that actively adapts to changing concentrations of external inorganic carbon (C\(_i\)). Under high C\(_i\) conditions, the cells probably rely on the passive diffusion of C\(_i\) into the chloroplast. Under low C\(_i\), on the other hand, the cells acquire the ability to actively accumulate C\(_i\) to support photosynthetic carbon fixation [1]. This C\(_i\)-concentrating mechanism (CCM) is observed within 2 h of transfer to low C\(_i\) [2].

The adaptation to low C\(_i\) is accompanied by the induction of several polypeptides, and it has been assumed that at least some of these are involved in the CCM. The first of these polypeptides to be cloned from *Chlamydomonas* was a periplasmic carbonic anhydrase [3]. Recently three other polypeptides were cloned. One of these encodes alanine-α-oxoglutarate aminotransferase [4] and another is a possible transmembrane protein [5]. The third polypeptide was identified as a β-carbonic anhydrase, and was found to be located in the mitochondria [6]. The exact role, if any, for these proteins in the CCM has not yet been established.

Little is known about the mechanism for the induction of genes by low C\(_i\) in *Chlamydomonas*. It has been suggested that changes in the accumulation of C\(_i\) or C\(_3\) metabolites may mediate the response [7]. A reduced supply of CO\(_2\) to ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the major carboxylating enzyme in photosynthesis, inevitably increases the rate of oxygenation by the enzyme, and leads to a burst in the production of glycollate [8]. *Chlamydomonas* has the ability to excrete excess glycolate, but the cells soon also acquire the capability to process more glycollate through the photorespiratory pathway [9]. Thus the lowering of the CO\(_2\) presumably changes the level of several of the metabolites in the photorespiratory pathway, and some of these changes may perhaps be utilized by the cell in a signal-transduction pathway. For the C\(_3\) metabolites, it is more difficult to predict how the metabolic intermediates are affected by the reduced carboxylation by Rubisco under low CO\(_2\). Little is also known about what short-term changes occur in the components of the light reaction, and also about how the levels of ATP and NADPH respond.

An alternative triggering mechanism by which the cells have the ability to sense the CO\(_2\) concentration in a more direct way, e.g. by a mechanism independent of the photosynthetic reactions, should also be considered. However, it has been observed that CCM is not induced in photosynthesis mutants [7]. Likewise, induction of the gene for the periplasmic carbonic anhydrase, *cah1*, is inhibited by 3-(3,5-dichlorophenyl)-1,1-dimethylurea (which inhibits photosynthetic electron transport) and by darkness [10], but it has also been reported that light is not always absolutely required for the induction of this gene, e.g. when the cells are grown in synchronous cultures [11]. The picture is further complicated by the observation that blue light is required for the induction of *cah1* [12].

In the present paper, we characterize two genes encoding mitochondrial β-carbonic anhydrase, one of the proteins that are induced by low CO\(_2\) [6]. By using a reporter gene system, we show that promoter elements from the genes are able to mediate a CO\(_2\)-regulated expression. We also present evidence that light regulates the promoter, and that photorespiration is not important for the induction process.

**MATERIALS AND METHODS**

**Culture conditions**

*Chlamydomonas reinhardtii* mutant strain CC425 (*cw15 arg2*) was obtained from the Chlamydomonas Genetics Center (Duke University, Durham, NC, U.S.A.). The cells were grown in continuous light (150 \(\mu\)mol/m per s) at 25 °C and in minimal

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**Abbreviations used:** Ars, arylsulphatase; CCM, C\(_i\)-concentrating mechanism; C\(_i\), inorganic carbon; XSO\(_4\), 5-bromo-4-chloro-3-indolyl sulphate; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase.

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The nucleotide sequences for the *ca1* and *ca2* genes have been submitted to the GenBank database with the accession numbers U80804 and U80805 respectively.
medium as previously described [13], except that the medium was supplemented with sodium acetate (200 mg/l), and arginine (50 mg/l) when required. For high-CO₂ conditions, the cultures were bubbled with air containing 5% CO₂. For low-CO₂ conditions, the cultures were either bubbled with air or not bubbled at all. Irradiance from the fluorescent lamps used as the light source was measured with a LI-185a quantum meter (Li-Cor Inc).

Gene isolation and Southern-blot analysis

A Chlamydomonas (cv 15) genomic library prepared in the cosmid vector pPR691 [14] was obtained from Saul Purton (University College London, U.K.). Three independent clones were isolated from the library after screening approx. 10000 colonies by hybridization using the previously described cal cDNA [6] as a probe. Restriction fragments from one of these clones were subcloned into pUC19, and the sequences were determined by automated DNA sequencing of both strands. Preparation of CsCl-purified genomic DNA from Chlamydomonas CC425 was as described by Weeks et al. [15] and modified by Rochaix et al. [16]. The Southern-blot technique, as well as other molecular-biology techniques, were essentially as described by Sambrook et al. [17].

Preparation of antibodies and Western-blot analysis

Antibody was raised against the mitochondrial carbonic anhydrase expressed in Escherichia coli by the following procedure. A DNA fragment containing the cal cDNA coding region and 152 bp of 3’ untranslated region was obtained by PCR using the primers 5’-GGCAATTCATCGAGGG-CCGCTCTACGACCCCACTCCC-3’ and 5’-TTTCTAGACGCGTAGGGTGCGGTG-3’. The amplification product was cleaved with EcoRI and XbaI and inserted into pMAL-c2 (New England Biolabs), downstream and in-frame with the gene encoding maltose-binding protein, malE. The resulting plasmid was transformed into the Escherichia coli strain DH5α. Cells were grown at 37°C to A₆₀₀ = 0.6, and expression was induced with isopropyl thio-β-D-galactoside (final concentration 1.5 mM). After 2 h, the cells were harvested by centrifugation, resuspended in 50 ml of 20 mM Tris/HCl, pH 7.4, containing 200 mM NaCl and 1 mM EDTA (column buffer), and disrupted in a precooled French pressure cell. Intact cells and membrane fractions were removed by centrifugation and the supernatant was diluted five times in column buffer and loaded on an amylose-resin column. The column was washed extensively with column buffer and the fusion protein was eluted using column buffer containing 10 mM maltose. The protein-containing fractions were concentrated using Centriprep 10 (Amicon) and buffer containing 10 mM maltose. The protein-containing fractions were concentrated using Centriprep 10 (Amicon) and buffer containing 10 mM maltose. The protein-containing fractions were concentrated using Centriprep 10 (Amicon) and buffer containing 10 mM maltose.

Plasmid construction

Putative promoter elements from the cal gene were inserted upstream of the promotorless arylsulphatase (Ars) gene [20] in the plasmid pJD54 obtained from John Davies (Carnegie Institution of Washington, Stanford, CA, U.S.A.). To isolate the upstream promoter region, an approx. 700 nt DNA fragment was amplified by PCR with a plasmid containing the cal gene as template, and by using the primer 5’-AGCCGTCGACAGGTTGCGGAATTCATCGAGGG-CCGCTCTACGACCCCACTCCC-3’ (binds in the 5’-most exon, upstream of the start codon) in combination with a M13 reverse primer (binds to the vector, upstream of the promoter). The amplification product was cleaved with SaI, and the resulting 464 nt DNA fragment (SaI cleaves in the promoter region, see Figure 1A) was inserted in the SaI site of pUC19 for control sequencing. The SaI fragment was cleaved out again and inserted in the proper orientation to the SaI site of the plasmid pJD54 to create pCAPars. To construct the plasmids pCAP2ars, pCAP4ars and pCAP8ars, a similar procedure was used. The downstream primer used now was 5’-GGCGTACCAAGGTATGGCGGAATTCATCGAGGG-CCGCTCTACGACCCCACTCCC-3’. For pCAP2ars, the upstream primer was 5’-GGCGTACCAAGGTATGGCGGAATTCATCGAGGG-CCGCTCTACGACCCCACTCCC-3’. For pCAP4ars, the downstream primer was 5’-GGCGTACCAAGGTATGGCGGAATTCATCGAGGG-CCGCTCTACGACCCCACTCCC-3’. For pCAP8ars 5’-GGCGTACCAAGGTATGGCGGAATTCATCGAGGG-CCGCTCTACGACCCCACTCCC-3’. The KpnI-cleaved amplification products were first cloned into pUC19 for control sequencing, and then into the KpnI site of pJD54 in the appropriate orientation.

Transformation of Chlamydomonas

Plasmids were introduced into Chlamydomonas strain CC425 by co-transformation with pARG7.8, a plasmid containing the selectable marker gene encoding arginosucinyl lyase [21]. The glass-bead transformation method used was described by Kindle [22] and modified by Purton and Rochaix [23]. For both plasmids pARG7.8 and the test constructs, 2 μg of DNA was used in the transformation. Arg² transformants of cells co-transformed with pARG7.8 and the test constructs were screened by in vivo staining for secreted Ars activity (see below). Transformants that appeared to express high levels of Ars activity were selected for further analysis in liquid cultures.

Ars assay

Ars activity was assayed essentially as described by Davies et al. [20]. Petri dishes with transformants growing on solid medium were overlaid with 2.5 ml of 0.3% melted agar in medium (at 45°C) containing 0.5 mM 5-bromo-4-chloro-3-indolyl sulphate (XSO). After 20 h, transformants expressing Ars were identified by a blue halo around the colonies. For liquid cultures, the cells were first pelleted by centrifugation for 1 min in a micro-centrifuge. XSO was added to the supernatant to a final concentration of 0.3 mM. After 4-18 h at 37°C, the appearing blue colour was quantified by measuring A₅₄₆. Alternatively, the assay was performed in a microtitre plate. The blue colour in the wells was then quantified by scanning the entire plate with a Pharmacia LKB ImageMaster DTS using a red/green filter. The level of Ars was represented by the absorbance values from the scan.

RESULTS

Isolation of the cal1 and cal2 genes

Previously, two very similar cDNA sequences (96% identity) for a mitochondrial carbonic anhydrase were obtained from Chlamydomonas [6]. The sequence analysis indicated that the two
cDNAs were transcribed from two separate genes, which were designated ca1 and ca2. In this study, we have used these cDNA sequences to isolate corresponding genomic sequences.

Three cosmid clones were obtained by screening a library, prepared with genomic DNA from Chlamydomonas [14], with radioactively labelled ca1 cDNA [6] as the hybridization probe. DNA prepared for each of the clones was digested with various restriction enzymes, and the restriction fragments were analysed by Southern blot, using ca1 cDNA as probe. When the restriction enzyme EcoRI was used, only a single 3 kb fragment was detected for all three clones. However, a more detailed restriction analysis indicated that, for two of the clones, two similar DNA fragments were contained within the 3 kb band. The 3 kb band was subcloned to the plasmid pUC19. A number of the subclones were analysed and found to contain two classes of inserts. By sequence analysis (see below), we found that these two classes corresponded to the ca1 and ca2 genes.

The Southern-blot analysis of the cosmids clones also detected a 3.5 kb band for two of the clones with BamHI. For the third clone, this band appeared to be truncated. When this 3.5 kb band was subcloned and subjected to sequencing from the ends, we found that it contained carboxic anhydrase sequences in an inverted repeat. From the orientation of the sequences, we concluded that this fragment contained the upstream gene regions for both ca1 and ca2. A restriction map based on the two 3 kb EcoRI bands and the overlapping 3.5 kb BamHI fragment is presented in Figure 1(A).

To investigate the possibility of more sequences with similarity to the ca1 and ca2 cDNAs, Chlamydomonas genomic DNA was subjected to Southern-blot analysis using ca1 cDNA as the probe. In the blot (Figure 1B), bands corresponding to the 3 kb EcoRI and 3.5 kb BamHI fragments of the cosmid clones can be identified. Apart from these, there is an additional approx. 2.4 kb EcoRI band. Since this is the only band on the genomic Southern blot that is not compatible with the restriction map prepared for the cosmid clones, we believe that it has arisen as a result of natural polymorphism. On the basis of the nucleotide sequences (below), one nucleotide change in either of the ca1 and ca2 genes could, at several locations, potentially create a new EcoRI site, giving rise to this extra band. We conclude that there is probably not more than two genes in the Chlamydomonas genome hybridizing to the ca1 and ca2 cDNAs, but the possibility for more genes cannot be excluded.

**Nucleotide sequence analysis of ca1 and ca2 genes**

Complete nucleotide sequences for the two 3 kb EcoRI fragments were determined by using custom-made oligonucleotide primers. For the 3.5 kb BamHI fragment, further subcloning was required to resolve the sequences for the inverted repeats. Put together, the sequences for the two genes cover 6691 nt of DNA, with restriction sites in agreement with the restriction map in Figure 1(A).

For each of the two genes, six exons and five introns could be recognized. For one of the genes, the sequences of the exons match exactly the ca1 cDNA sequences [6], and we conclude that this must be the ca1 gene. The other gene contained sequences that were identical with the ca2 cDNA sequences, and was hence designated ca2. The overall structure of the two genes is depicted in Figure 1(A), and the full sequences are presented in Figure 2.

The exon/intron/exon borders in the two genes are conserved. All sites conform to the consensus sequence G/GTXXXG . . . . . . AG/C/G (I denotes the splice site), which is in agreement with the universal splice sites for nuclear-encoded eukaryotic genes [24]. The five introns in each of the genes are, as commonly found in Chlamydomonas, fairly small (121–261 nt long). There are only minor differences between the two genes in the length of the introns, and the sequences of the introns are also very similar. The percentage of identical nucleotides is about the same as for the exons (96%), but the majority of the differences for the exons are in the 3′ untranslated region. The protein-coding sequences for the two genes are 98.9% identical.

The ca1 and ca2 genes were found to be located ‘head to head’ in a large inverted repeat. The distance between the 5′-most exons for the two genes is about 2 kb. The entire region is not repeated; about 500 nt in the middle of the region consists of unique sequence (see Figure 1A). The region between the 5′-most exons presumably contains the cis-acting DNA elements that regulate the transcription of the genes. We have, however, not been able to distinguish any typical promoter/enhancer elements. The genes apparently belong to a group with TATA-box-less promoters. Furthermore, when the sequences were compared with the gene encoding periplasmic carboxic anhydrase in Chlamydomonas [3], which is also regulated by the CO₂ concentration, no marked similarities were detected. This lack of similarity between the two promoters may indicate that there are differences in the mode of regulation. Alternatively, the similarity searches were not sensitive enough to single out functional homologies, or maybe the regulatory sequences lie outside the sequenced regions. The promoter sequences for the ca1 and ca2 genes were found to be remarkably well conserved. The 500 nt
Figure 2  For legend see opposite page.
corresponding region of probably signifying the functional importance of these regions.

The full sequence of the ca1

Figure 2 Nucleotide sequences for the ca1 and ca2 genes

The full sequence of the ca1 gene is shown, and the differences in the ca2 gene are indicated below. Sequences corresponding to the ca1 and ca2 cDNAs are printed in upper-case letters, introns and downstream sequences (three nucleotides only) are in lower-case letters, and upstream sequences are in italics. The start and stop codons are underlined.

relative expression levels in cells transformed with pCAPars and pJD54, the two cultures were grown in parallel at approximately the same densities (about 5 × 10^6 cells/ml). The supply of 5% CO2 in the culture was then turned off, and the cultures were instead bubbled with air. Samples were removed from the culture before and after induction. The supernatants were assayed for accumulation of Ars activity and, for the pCAPars transformants, the cells were analysed for expression of the original Ars activity before and after induction, and the accumulated activity increased all the way up to 9 h after induction. In cells transformed with pJD55, Ars was constitutively expressed before and after induction, and the accumulation led to a linear increase in activity.

Regulation by light and O2

To determine the light requirement for the induction of the promoter, the cells transformed with pCAPars were grown in the light at high CO2, pelleted by centrifugation, and resuspended at approx. 10^7 cells/ml. The culture was then split into 2 ml glass tubes and incubated at different irradiances for 4 h. The vials were agitated occasionally during the incubation to prevent sedimentation of the cells. Ars activity was determined in the supernatants after centrifugation. The results (Figure 4) show that there was no detectable induction at irradiance at or below 75 µmol/s/m^2. From 100 µmol/m^2 per s and up to 500 µmol/m^2 per s there was a dramatic increase in Ars activity, but above 500 µmol/m^2 the activity levelled off.

To study the effect of O2 on the induction of the ca1 promoter, a culture of pCAPars transformants, grown at high CO2 to a density of approx. 10^7 cells/ml, was bubbled with a gas mixture containing an air level of CO2 but only 0.12% O2. Under these conditions, Ars activity was measured for 9 h after transfer to low CO2. The accumulation was assayed in the supernatants before and after induction. The results (Figure 5) show that there was no detectable induction of Ars activity at any of the irradiances tested. Furthermore, the amount of protein was still increasing substantially from 6 to 9 h. Ars activity in the same cells (Figure 3B) was first detected 3 h after transfer to low CO2, and the accumulated activity increased all the way up to 9 h after induction. In cells transformed with pJD55, Ars was constitutively expressed before and after induction, and the accumulation led to a linear increase in activity.

Regulation of carbonic anhydrase gene expression in Chlamydomonas

The full sequence of the ca1 gene is shown, and the differences in the ca2 gene are indicated below. Sequences corresponding to the ca1 and ca2 cDNAs are printed in upper-case letters, introns and downstream sequences (three nucleotides only) are in lower-case letters, and upstream sequences are in italics. The start and stop codons are underlined.

Protein analysis with the Ars reporter gene

To study transcriptional regulation, a 381 nt putative promoter element, derived from the ca1 gene but 100% identical with the corresponding region of ca2, was fused upstream of the promoterless ars gene [20] to generate the test construct pCAPars. The construct was transformed into Chlamydomonas (cw15 arg2) by co-transformation with the selectable marker pArg7.8. The plasmid pJD55, with the β-tubulin promoter fused upstream of the promoterless ars gene [20], was also transformed into Chlamydomonas as a positive control. Transformants expressing the reporter gene were screened for by in situ staining of the Arg+ colonies. A few percent of the colonies had an apparently high level of Ars expression, and they were selected for analysis in liquid culture.

Ars activity was determined for cultures of the transformed Chlamydomonas after transfer from high to low CO2. To compare immediately upstream of the 5′-most exons are 100% identical, probably signifying the functional importance of these regions.
Table 1  Induction of the cat1 promoter in low O2

Chlamydomonas transformed with pCAPArs was grown in high CO2 to a density of approx.
10⁷ cells/ml, and split into nine cultures. The cultures were bubbled with three different gas
mixtures, and incubated at three different irradiances. The cells were pelleted after 4 h of
incubation, and the accumulation of Ars activity in the supernatants was determined. The relative
accumulation of Ars is presented as A660 values.

<table>
<thead>
<tr>
<th>Gas mixture</th>
<th>Irradiance (µmol/s per m²)</th>
<th>Ars activity (OD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03% CO2, 21% O2 (air)</td>
<td>50</td>
<td>0.00 0.46 0.93</td>
</tr>
<tr>
<td>0.03% CO2, 15% O2</td>
<td>150</td>
<td>0.00 0.39 1.12</td>
</tr>
<tr>
<td>5% CO2, 21% O2</td>
<td>1400</td>
<td>0.00 0.00 0.00</td>
</tr>
</tbody>
</table>

* Prepared by diluting 5% CO2 (in air) with N2.

Promoter deletions

The 381 nt promoter element used in the studies above was sufficient to regulate the accumulation of Ars in a CO₂-dependent manner. Three new constructs, with promoters extending to a different extent up into the upstream region of the cat1 gene, were prepared. pCAP8ars contained 804 nt of upstream sequence (from nt 175 in Figure 2), pCAP4ars contained the same 381 nt promoter element as pCAPars (from nt 599) and pCAP2ars contained only the 194 nt closest to the start of transcription (from nt 786). The three constructs were transformed into Chlamydomonas by co-transformation with pARG7.8. The Arg⁺ colonies were screened for Ars activity, and the frequency of blue to non-blue colonies was determined. For pCAP2ars, the frequency was found to be 2.5%, (19/750), for pCAP4ars 1.6%, (11/700) and for pCAP8ars 2.0%, (18/900). The control plasmids pJD54 (without promoter) and pJD55 (with the β-tubulin promoter) were transformed to the same experiment, and the frequencies for these plasmids were 0.0% (0/900) and 0.4% (4/1000) respectively. Assuming that the frequency of co-transformation was similar for all the plasmids, and that genes with strong promoters have a higher chance of being expressed after transformation into Chlamydomonas, these findings indicate two features of the cat1 promoter under the conditions used: (i) the promoter strength is approximately the same when 194, 381 and 786 nt of the upstream sequence is included; (ii) the promoter has a high level of activity compared with the β-tubulin promoter. Transformants with the plasmids pCAP2ars and pCAP4ars were also tested for induction in liquid culture. Figure 5 shows that the induction of Ars activity from these two constructs is similar to induction from pCAPars (Figure 3), except that now induction was detected within 2 h instead of 3 h. The similar Ars expression pattern for the two constructs confirms that the 194 nt cat1 promoter in pCAP2ars is sufficient to govern both a full level of transcription and a firm regulation by the external CO₂ concentration.

DISCUSSION

The main motivation for isolating the Chlamydomonas genes encoding β-carbonic anhydrase was the observation that the corresponding transcripts appeared when the cells were transferred from high- to low-CO₂ conditions [6]. This gene activation, which most likely happens at the transcriptional level, occurs simultaneously with the induction of the CCM. Independent of a possible role for the genes in the CCM, characterization of the factors activating the expression of these genes will provide information on the metabolic adjustments that occur when cells are transferred from high- to low-CO₂ conditions.

The cat1 and ca2 genes were found to be organized as an inverted repeat (Figure 1A). Previously, two periplasmic carbonic anhydrase genes were found in a repeat [10], so the occurrence of duplicated genes is probably not uncommon in Chlamydomonas. The high level of overall sequence identity (97%) between the two genes (Figure 2) suggests a recent duplication. There was only one difference in the deduced amino acid sequences, located in the tentative presequence. The upstream regulatory sequences were also very similar: the 381 nt and 194 nt promoter elements examined in this study were 100% identical for the two genes. Combined with the finding that both genes were expressed under low CO₂, but not high CO₂ [6], these observations indicate that there are no functional differences between the cat1 and ca2 genes. The effect of the gene-duplication event is therefore probably just an increase in the total quantity of the mitochondrial β-carbonic anhydrase.

The notion that the external CO₂ concentration can affect the transcription of genes was verified by using the Ars reporter gene system. The insertion of upstream sequences from the cat1 gene could govern CO₂-conditioned expression of the promoter-truncated ars gene that was similar to the endogenous cat1 and ca2 genes (Figure 3). The finding that 194 nt of promoter sequence is sufficient for stringent repression of transcription at high CO₂, as well as for facilitating the apparently high level of transcription...
at low \( \text{CO}_2 \) (Figure 5), will help in the identification of the cis-
acting factors involved in the regulation.

The only \( \text{CO}_2 \)-regulated gene previously described in
\textit{Chlamydomonas} is \textit{cah1}, encoding a periplasmic carbonic
anhydrase [10]. When the upstream DNA sequence of \textit{cah1} was
compared with the \textit{cal1/cal2} sequences, no similarities were
detected. This could indicate a difference in the mode of
regulation, but such a presumption is not supported by the
experimental data. Besides the induction by low \( \text{CO}_2 \) that the two
genes have in common, both genes appear to be affected by the
light conditions. Neither \textit{cah1} [10] nor \textit{cal} was expressed when
light-adapted cells were transferred to low \( \text{CO}_2 \) in the dark. For the
\textit{cal} gene we here report (Figure 4) that at least 75 \( \mu \text{mol} \) per
\( \text{m}^2 \) was required for detectable expression within 4 h. Further-
more our data establish that light is not only a requirement
for the induction, but also is a key regulator of the promoter.
Between 100 and 500 \( \mu \text{mol} \text{s}^{-1} \text{per m}^2 \), transcriptional activity of the
promoter was strictly dependent on the level of irradiance.

The similarity between the curve in Figure 4 and a light-response
curve for \( \text{O}_2 \) evolution in \textit{Chlamydomonas} [25] suggests that the
regulation of the promoter is tightly coupled to photosynthesis.

We have also compared the DNA sequences of the \textit{cal1/cal2}
promoters with motifs of other light-regulated promoters [26],
but were not able to detect any revealing similarities.

The availability of transgenic \textit{Chlamydomonas} cells expressing
an easily assayed reporter gene under control of a \( \text{CO}_2 \)-regulated
promoter will greatly facilitate studies on the transduction
pathway. The cells will simplify the search for the metabolic
conditions that trigger the response. We show in this study
(Table 1) that metabolites in the photosynthetic pathway probably
do not trigger the response. The transgenic cells will also facilitate the identification of genes of relevance. We are in
the process of mutagenizing the cells in an attempt to isolate
genes in the transduction pathway. The gene-tagging technique
[27], combined with the \textit{in vivo} detection of Ars to screen mutant
colonies, has previously been used to clone new genes in
\textit{Chlamydomonas} [28].

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