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The Cytochrome *c* Gene from the Green Alga *Chlamydomonas reinhardtii*. Structure and Expression in Wild-Type Cells and in Obligate Photoautotrophic (dk) Mutants

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The expression of the *Chlamydomonas reinhardtii* cytochrome *c* gene was studied at the steady-state mRNA level. The inclusion of acetate under illumination produced a marked increase in cytochrome *c* transcripts. This effect was not affected by two inhibitors of mitochondrial energy metabolism. Three different obligate photoautotrophic mutants with defective mitochondria showed normal levels of induction, suggesting that utilization of acetate for respiration is not required for this process. Light, in the presence or absence of acetate, also promoted an increase in cytochrome *c* transcript levels. This effect could be abolished by treatment of the cells with an inhibitor of the photosynthetic electron transport chain, suggesting that light acts through photosynthesis to promote the induction. In addition, a genomic clone encompassing the *Chlamydomonas* cytochrome *c* gene has been isolated and analyzed. The gene contains three introns, two of which are located at positions similar to those in the rice and *Arabidopsis* cytochrome *c* genes, indicating the existence of an evolutionary link. It is concluded that the cytochrome *c* gene from *C. reinhardtii* is subject to metabolic regulation through a mechanism that responds to the intracellular level of either acetate or a compound derived from its metabolization through a pathway different from mitochondrial respiration.

Key words: *Chlamydomonas reinhardtii* — Cytochrome *c* — Exon/intron structure — Gene expression — Mitochondrion.

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; NMR, nuclear magnetic resonance; TCA, tricarboxylic acids.

The nucleotide sequence reported in this paper has been submitted to the EMBL, GenBank and DDBJ under accession number Z99829.

Introduction

The study of the expression of genes encoding mitochondrial components is particularly attractive, since the different proteins that compose the organelle are encoded by two separate genomes. This raises the question of how the coordinated expression of all components is achieved. In most organisms studied, mitochondrial biogenesis is a regulated process. In yeasts, for example, the kind and amount of respiratory substrates, as well as O_2 concentration, influence the rate of synthesis of respiratory chain components (de Winde and Grivell 1993, Grossman and Lomax 1997). In mammals, tissue and developmental stage-specific regulation have been observed (Capaldi 1990, Grossman and Lomax 1997).

In phototrophic organisms, it is likely that the biogenesis of mitochondrial components involved in respiration is subject to specific modes of regulation, given the presence of a particular metabolism (photosynthesis) that is involved in the production of energy and organic carbon compounds. Several studies have shown that plant mitochondrial components encoded by both genomes are expressed in a tissue-specific manner (Huang et al. 1994, Smart et al. 1994, Heiser et al. 1996, Li et al. 1996). Other forms of regulation, however, have not been described, except for the alternative oxidase, which is involved in cyanide-insensitive respiration (Vanlerberghe and McIntosh 1997).

Since in other systems the expression of the cytochrome *c* gene is correlated with the expression of other components of the respiratory chain (de Winde and Grivell 1993, Grossman and Lomax 1997), we have chosen this gene to analyze the modes of regulation that operate in photosynthetic organisms. Recently, we have demonstrated that the sunflower nuclear gene encoding mitochondrial cytochrome *c* is regulated by both tissue type and environmental factors, such as light, nitrate, and carbon source (Felitti et al. 1997, Felitti and Gonzalez 1998). We have proposed that these effects are most likely related to metabolic changes that occur within cells upon illumination or nitrate influx or reduction (Felitti and Gonzalez 1998). A sort of metabolic regulation of the cytochrome *c* gene has also been suggested in studies with the unicellular green alga *Chlamydomonas reinhardtii*, whose most recent common ancestor with higher plants has lived several hundred million years ago (Amati et al. 1988). These authors observed that cytochrome *c* mRNA levels are higher in cells grown with acetate in the culture medium (Amati et al. 1988).

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To gain insight into the factors and mechanisms that govern cytochrome *c* gene expression in *C. reinhardtii*, in the present work we have isolated a genomic clone encoding this protein and studied its expression. We conclude that this gene is subject to metabolic regulation through products derived from photosynthesis and acetate incorporation. Induction by acetate does not require its metabolization for respiratory purposes.

Materials and Methods

Cell culture conditions

Chlamydomonas reinhardtii (strain cc 124 mt^-) cells (considered wild type), and acetate non-utilizing mutants, obtained from the Chlamydomonas Genetics Center (Duke University, U.S.A.), were grown under constant light (70 μmol m^{-2} s^{-1} photosynthetic photon flux density provided by cool white fluorescent lamps) in Tris-minimal medium (Harris 1989) up to the mid exponential phase (approximately 2×10^6 cells ml^{-1}), when the different treatments were initiated. Mutants were checked for absence of growth in the presence of acetate in the dark before utilization. They were maintained in minimal medium to avoid any selective advantage of revertants.

Treatment conditions

The different treatments were applied to cells in culture medium at the density described above. Sodium acetate was used at a concentration of 17.4 mM (pH 7.0) and was added directly to Tris-minimal medium. When the effect of light was tested in the presence of acetate, this compound was added 24 h before the different treatments, and cells were kept in complete darkness during this period. DCMU, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), and antimycin A were dissolved in ethanol and used at a concentration of 4 μM, 1 μM, and 0.5 μM, respectively. In all cases, a similar amount of the corresponding solvent was added to controls.

Nucleic acid isolation and analysis

Cells were harvested by centrifugation (5,000 × *g*) and resuspended in 50 mM Tris-HCl (pH 8.0), 300 mM NaCl, 5 mM EDTA. Total RNA was extracted several times with phenol/chloroform in the presence of 20 mM aurintricarboxylic acid and precipitated with ethanol. This material was resuspended in H_2O and subjected to a new precipitation step with 2 M LiCl. For Northern blot analysis, specific amounts of RNA were electrophoresed through 1.5% (w/v) agarose/6% (v/v) formaldehyde gels. The integrity of the RNA and equality of RNA loading were verified by ethidium bromide staining. RNA was transferred to Hybond-N nylon membranes (Amersham Corp.) and hybridized overnight at 65°C to a ^{32}P-labelled cytochrome *c* probe (617-bp *Sal*I/*Eco*RI fragment from cDNA clone C321 (Amati et al. 1988), a kind gift of Dr. Michel Goldschmidt-Clermont, University of Geneva, Switzerland) in buffer containing 6× SSC (1× SSC is 0.15 M NaCl, 0.015 M Na_3-citrate, pH 7.0), 0.1% (w/v) polyvinylpirrolidone, 0.1% (w/v) BSA, 0.1% (w/v) Ficoll and 0.2% (w/v) SDS. Filters were washed with 2× SSC plus 0.1% SDS at 65°C (four times, 15 min each), 0.1× SSC plus 0.1% SDS at 37°C during 15 min, dried and exposed to Kodak X-AR films. To check the amount of total RNA loaded in each lane, filters were then re-probed with a 25S rDNA from *Vicia faba* under similar conditions as those described above, except that hybridization was performed at 62°C and the wash with 0.1× SSC was omitted. Autoradiographs were scanned using a Shimadzu densitometer and values (arbitrary units) were corrected with those obtained with the rDNA probe. The experiments shown are representative of the results obtained in other, similar, experiments.

Southern blot analysis

Total DNA was extracted essentially as described by Rochaix et al. (1987) The DNA was cut with restriction enzymes, separated on 0.7% agarose gels, transferred with 0.4 M NaOH, and hybridized with different probes essentially as described for Northern analysis.

Genomic library screening

The *C. reinhardtii* genomic library constructed in λEMBL3 was provided by Dr. Michel Goldschmidt-Clermont, University of Geneva, Switzerland. For the isolation of clones containing the cytochrome *c* gene, the cDNA probe described for Northern analysis was used to screen 2×10^5 p.f.u. from the library. Phage DNA was transferred to Hybond-N. After overnight hybridization, filters were washed and exposed to X-ray films. Positive clones were purified through successive rounds of plating and hybridization. Purified clones were used to isolate phage DNA (Kaslow 1986).

Recombinant DNA techniques and DNA sequencing

Recombinant DNA techniques were performed using standard protocols (Ausubel et al. 1987). Phage inserts were analyzed by restriction enzyme treatments followed by Southern blots. Subclones were made in pUC119 or pBluescript SK^- phagemids. DNA sequencing was performed by the dideoxy chain termination method (Sanger et al. 1977) using the fmol sequencing kit (Promega Corp.). Sequencing reactions were performed on both strands using double-stranded DNA and plasmid- or insert-specific primers.

Nuclear magnetic resonance (NMR) analysis

To analyze the fate of acetate in wild-type and mutant strains, [2-^{13}C]acetate (99 atom % ^{13}C, Sigma Chemical Co.) was included in the culture medium. After 24–48 h, cells were harvested, and perchloric acid extracts were obtained essentially as described (Thomas and Baxter 1987). ^{13}C NMR spectra were measured in D_2O solutions, in a Bruker AC-200 spectrometer, using standard conditions (SI = 64 K, repetition time: 1.5 s, with a 33 *f* pulse) on 6.000 transients.

Results

Regulation of cytochrome c gene expression

In a previous study, Amati et al. (1988) have demonstrated that the steady-state level of cytochrome *c* mRNA is higher in *Chlamydomonas* cells grown in the presence of acetate than in photoautotrophically grown cells. Fig. 1 shows the time-dependent induction of cytochrome *c* gene expression promoted by the addition of acetate to the culture medium under illumination. A significant increase in transcript levels is already noticeable after 2 h of incubation with acetate, indicating the existence of regulation of the expression of the cytochrome *c* gene by this compound, or by metabolic changes that occur upon its incorporation from the culture medium.

To gather information about the nature of the effect of acetate on cytochrome *c* gene expression, cells were treated with either the uncoupler CCCP, the electron transport chain inhibitor antimycin A, or both (Fig. 2). The presence of these compounds did not significantly affect the increase in cytochrome *c* mRNA levels promoted by acetate. This may indicate that the effect of acetate is not exerted through its utilization by the mi-

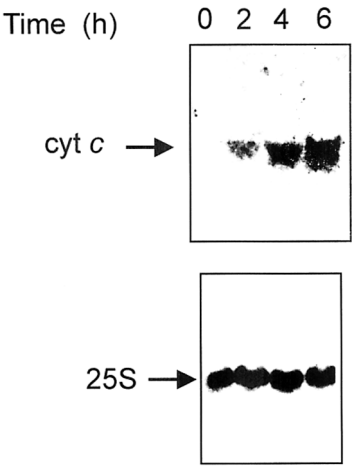

Fig. 1 Effect of acetate on cytochrome *c* transcript levels. Cells grown in minimal medium under illumination were incubated for the times indicated under similar conditions with the addition of 17.4 mM NaOAc. After incubation, cells were harvested and RNA (10 μg) was analyzed by Northern blot as described in Materials and Methods.

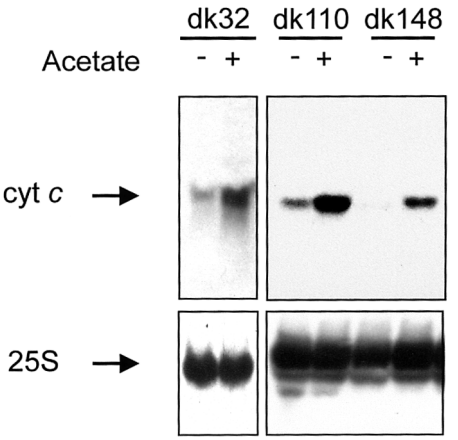

Fig. 3 Effect of acetate on cytochrome *c* gene expression in obligatory photoautotrophic mutants. Different *dk* mutant cells grown in minimal medium under illumination were incubated for 2 h under similar conditions with (+) or without (–) the addition of 17.4 mM NaOAc. After incubation, cells were harvested and RNA (10 μg) was analyzed by Northern blot as described in Materials and Methods.

tochondrial respiratory chain. To further analyze this possibility, we have studied the effect of acetate in obligate photoautotrophic (*dark-dier*) mutants which show defects in acetate utilization and mitochondrial structure (Wiseman et al. 1977). It is noteworthy that mutant *dk-32*, which possesses grossly altered mitochondria and highly reduced cytochrome *c* reductase and cytochrome *c* oxidase activities (Wiseman et al. 1977), showed normal levels of acetate induction (Fig. 3). This agrees with the results obtained using inhibitors, and supports the view that functional mitochondria are not required for the induction of the cytochrome *c* gene by acetate. Accordingly, two other mutants with less severe alterations, *dk-110* and *dk-148*, also displayed acetate induction (Fig. 3), although *dk-148* had lower levels of cytochrome *c* transcripts both in the presence and absence of acetate.

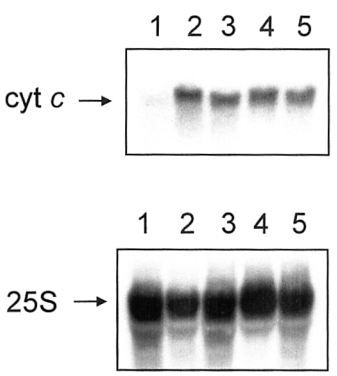

Fig. 2 Effect of CCCP and antimycin A on cytochrome *c* gene expression. Cells were incubated under illumination during 4 h either in the absence (*lane 1*) or presence (*lanes* 2–5) of 17.4 mM NaOAc, including 1 μM CCCP (*lane 3*), 0.5 μM antimycin A (*lane 4*), or both (*lane 5*). RNA (10 μg) was extracted and analyzed as described.

Acetate utilization by wild-type and the mutant strain *dk-32* was analyzed by NMR spectroscopy in extracts obtained after incubation of cells with ^{13}C-labelled acetate. In wild-type cells, label was progressively observed in intermediates of the tricarboxylic acids (TCA) cycle and amino acids derived from it, and then in carbohydrates, indicating the utilization of acetate for gluconeogenesis (Fig. 4A). Prolonged incubation in the presence of acetate produced a significant scrambling of the label, with multiply enriched compounds, including doubly-labelled acetate, as suggested by the presence of a doublet at the C-2 resonance position. For the mutant, the presence of ^{13}C-enriched TCA cycle intermediates was less evident, and some specific peaks were different from those obtained with the wild-type strain. A peak corresponding to succinate is prominent in this region (Fig. 4B). In contrast to wild type, higher proportions of labelled carbohydrates were observed, indicating that the mutant incorporates acetate mainly for gluconeogenesis. The presence of sharper peaks in the sample from the mutant strain probably reflects less randomization through the TCA cycle, which may not be completely operative in these cells due to defects in the respiratory chain. The accumulation of succinate may reflect a blockage at the level of succinate dehydrogenase, which is directly linked to the respiratory chain. As a whole, the results seem to exclude mitochondrial acetate utilization as a source of the signal leading to cytochrome *c* gene induction.

Since the results described above suggest the existence of metabolic regulation of the expression of the cytochrome *c* gene, we have also analyzed the effect of illumination on expression. For this purpose, cells adapted to acetate and darkness 24 h before the start of the experiment were illuminated,

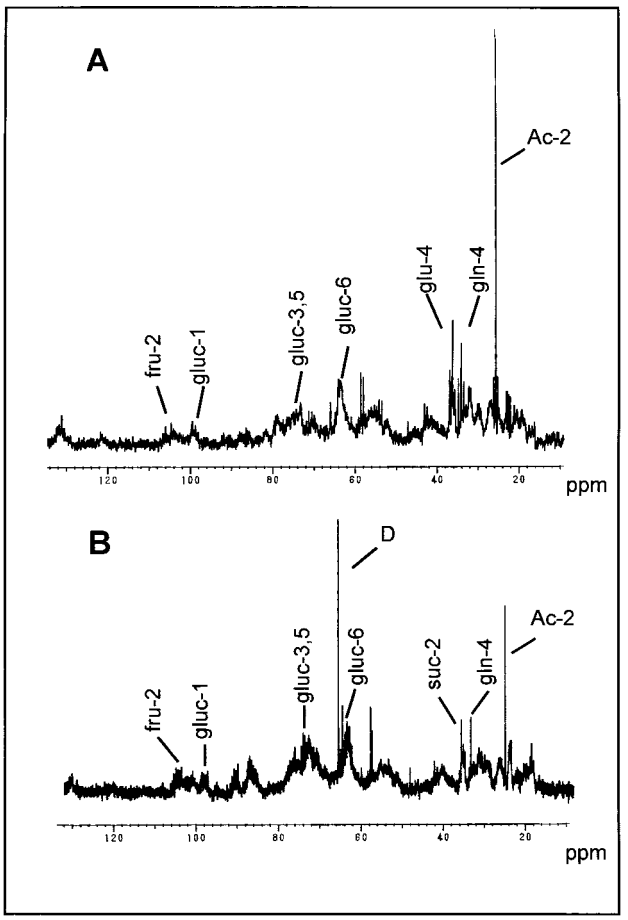

Fig. 4 NMR analysis of cell extracts obtained after incubation in the presence of ^{13}C-labelled acetate. Wild-type (A) and *dk-32* (B) mutant cells grown in minimal medium under illumination were incubated for 24 h under similar conditions in the presence of (2-^{13}C)acetate. After incubation, perchloric acid extracts were obtained and analyzed by ^{13}C-NMR as described in Materials and Methods. The resonance positions of known compounds are included for reference: *Ac-2*, C-2 of acetate; *glu-4* and *gln-4*, C-4 of glutamic acid and glutamine, respectively; *suc-2*, C-2 of succinate; *gluc-1*, *gluc-3,5* and *gluc-6*, C-1, C-3,5, and C-6 of glucose, respectively; *fru-2*, C-2 of fructose; *D*, dioxane included as standard.

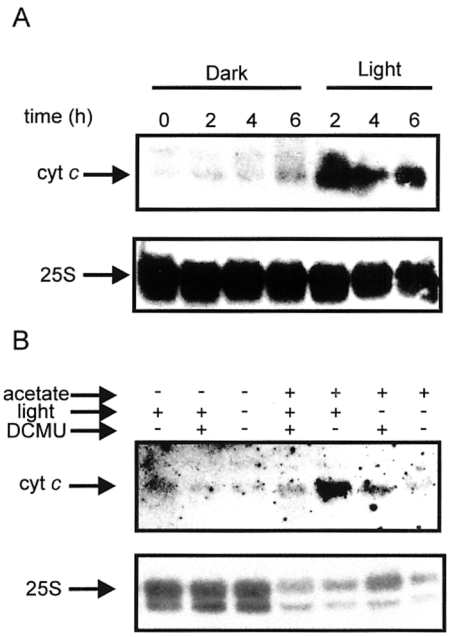

Fig. 5 Effect of light on cytochrome *c* transcript levels. (A) Cells adapted to darkness and acetate during 24 h were either transferred to light or kept in darkness. After different times, RNA (10 μg) was analyzed as described. (B) To analyze the effect of DCMU, either cells grown in minimal medium under constant illumination (*lanes* 1–3), or cells adapted to acetate and darkness during 24 h (*lanes* 4–7) were used. These cells were subjected to different light treatments in the presence or absence of DCMU (4 μM). After 4 h, RNA was extracted and analyzed; samples obtained in the presence of acetate contained less RNA than those obtained from minimal medium (10 μg and 20 μg, respectively).

and the corresponding steady-state mRNA levels were analyzed at different times (Fig. 5A). It can be observed that there is a considerable increase in mRNA levels upon illumination, while in cells that have been kept in darkness, only a slight increase is observed (Fig. 5A). A similar experiment conducted in minimal medium showed consistent results. In this case, cells grown under constant light were transferred to darkness and RNA extractions were performed at different times. A decrease with time of cytochrome *c* transcript levels in cells transferred to darkness could be observed (not shown), suggesting that the effect of illumination operates both in the presence or absence of acetate.

Light can act through either photosynthesis or the activation of a signalling pathway involving other photoreceptors. To discern which role light plays in the induction of cytochrome *c* mRNA, the effect of an inhibitor of the photosynthetic electron transport chain, DCMU, was analyzed. As shown in Fig. 5B, this compound completely abolished the activating effect of light in cells adapted to acetate and darkness. DCMU also promoted a decrease of transcript levels in cells grown in minimal medium under illumination, an effect similar to that observed when cells where transferred to darkness (Fig. 5B). This suggests that a photosynthesis-dependent mechanism is operating. It can be suggested that metabolic changes originated upon illumination are responsible for cytochrome *c* gene induction.

Isolation and characterization of a cytochrome c genomic clone

As a first step to characterize the molecular mechanisms that may act in the regulation of the expression of the only cytochrome *c* gene present in *C. reinhardtii*, we have screened a genomic library constructed in λEMBL3 and characterized clones encompassing this region. Particularly, an approximately 15-kbp clone has been analyzed, which comprises sequenc-

Fig. 6 Nucleotide sequence of the *Chlamydomonas reinhardtii* cytochrome *c* gene. The A from the methionine start codon was assigned number 1. *Arrows* specify the boundaries of introns. The initiation and termination codons, and the putative polyadenylation signal, are shown in *bold*. Four sequences that match the consensus for the tubulin box are *underlined.* A putative light-responsive heptamer element (CGCGCAC) is *doubly-underlined*. A repeated sequence found upstream from the initiation codon is *wavy-underlined*.

es 1.7-kpb upstream and 12-kbp downstream, respectively, from the 5′- and 3′-ends of the cytochrome *c* cDNA clone previously characterized (Amati et al. 1988). The cytochrome *c* gene contains three introns located at positions 43, 111, and 245 of the coding sequence (Fig. 6; we have assigned number 1 to the A of the methionine initiation codon). All the introns conform the 5′-GT...AG-3′ rule observed in nuclear introns of other organisms. Notably, two of the introns (numbers 2 and 3) are located at almost the same positions (1 bp upstream) as the two introns of the rice cytochrome *c* gene (Fig. 7A; Kemmerer et al. 1991), suggesting that these introns have been conserved through evolution from the last common ancestor about 700

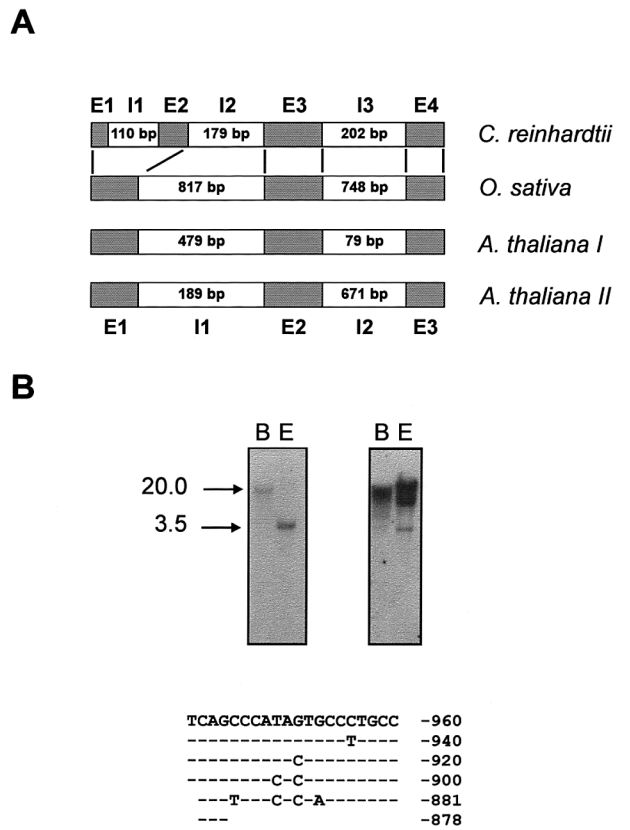

Fig. 7 (A) Exon/intron structures of the cytochrome *c* genes from *Chlamydomonas reinhardtii*, *Oryza sativa* (rice), and *Arabidopsis thaliana*. Shaded and white boxes indicate exons and introns, respectively. The introns within the plant genes are not drawn to scale. (B) Southern blot analysis of the genomic region upstream from the translation start site. Total genomic DNA digested with either *Bam*HI (*B*) or *Eco*RI (*E*) was probed with a *Sal*I/*Xho*II fragment comprising sequences from −731 to +8 (*left*), or a *Sau*3A fragment from −1,043 to −727 (*right*). Numbers indicate sizes in kbp. *Below*, an alignment of the repeated sequence is presented. Dashes indicate identical nucleotides.

million years ago. Indeed, a search for cytochrome *c* coding sequences in GenBank revealed two potential genes from *Arabidopsis thaliana* with introns at the same positions (accession numbers AF000657 and AL049487). Another interesting feature is that introns 1 and 3 of the *Chlamydomonas* gene begin with the same sequence GTAGGTTG.

Within the promoter region, four sequences that match 70% with the consensus for the tub box, a motif first described in the tubulin genes from *C. reinhardtii* (Brunke et al. 1984), and subsequently recognized in many other genes from this organism (Goldschmidt-Clermont and Rahire 1986, Williams et al. 1989, Curry et al. 1992, Müller et al. 1992), were found (Fig. 6). In addition, the sequence CGCGCAC, recently observed by Hahn and Kück (1999) in promoters of light-regulated genes, is present 865 bp upstream from the initiation codon. We have also identified a small repeated sequence about 900 bp upstream from this ATG (Fig. 6, 7B). Southern blots performed with an 800 bp fragment comprising sequences upstream from the initiation codon identified a single band, indicating that this region is present in a single copy within the *Chlamydomonas* genome (Fig. 7B, *left*). This result agrees with those obtained by Amati et al. (1988) using the cytochrome *c* cDNA as a probe. However, when a contiguous fragment comprising mainly the repeated sequence was used, several bands were observed, indicating that this sequence is present at different positions within the genome (Fig. 7B, *right*).

Discussion

We have observed that the carbon source modulates the expression of the cytochrome *c* gene, with acetate acting as an inducer. The effect of acetate has been studied in the presence of light, so that changes could be monitored at short times after the inclusion of acetate to cultures grown in minimal medium. Cells grown in acetate in the dark, however, have higher transcript levels than cells grown in minimal medium, indicating that acetate induces cytochrome *c* gene expression also in the absence of light. So far, we have only studied changes in steady-state mRNA levels. We have used antibodies raised against yeast cytochorme *c* to analyze the effect of acetate on protein levels, but failed to obtain reproducible signals in Western blots of whole cell protein extracts. Although it is likely that this is the case, we do not know, then, if the regulation reported here provokes the accumulation of higher protein levels in acetate-grown cells.

Chlamydomonas reinhardtii belongs to a group of organisms generally termed acetate flagellates, because they use almost exclusively this compound as carbon source under heterotrophic conditions (Harris 1989); that is, they cannot use carbohydrates, presumably because they are not able to incorporate them from the external medium. Acetate can act as a substrate for both respiration (through the TCA cycle) and the synthesis of carbohydrates (through the glyoxylate cycle). There are reports that acetate transiently inhibits photosynthesis (Endo and Asada 1996) and stimulates respiration in air-grown *Chlamydomonas* cells (Fett and Coleman 1994, Endo and Asada 1996). In addition, cells grown in the presence of acetate showed decreased levels of photosynthetic oxygen evolution and CO_2 fixation, although increased respiratory activity was not observed (Heifetz et al. 2000).

The induction of the expression of the cytochrome *c* gene by acetate reported here is not affected by the presence of two inhibitors of mitochondrial energy metabolism. We then conclude that utilization of acetate by the mitochondrial respiratory chain is not required for induction. Accordingly, three mutants unable to grow on acetate because they possess altered mitochondria still show induction by this compound. Since these mutants cannot use acetate for growth, this also rules out that the effect of acetate is due to a general induction of growth and

division.

dk mutants have a variety of alterations in mitochondrial ultrastructure and deficiencies in cytochrome *c* oxidase activity and, for *dk-32*, also in cytochrome *c* reductase activity (Wiseman et al. 1977). The three selected mutants also show different rates of acetate incorporation (similar, lower, and higher than wild-type for *dk-32*, *dk-110*, and *dk-148*, respectively) and normal isocitrate lyase activity (Wiseman et al. 1977). There seems to be no relationship, then, between the rate of acetate incorporation and cytochrome *c* gene induction. This may indicate that acetate itself is not the actual inducer, and that its metabolization through a defined pathway is required. In this sense, it is noteworthy that *dk-32*, which directs most of the incorporated acetate to the synthesis of carbohydrates, still shows acetate induction. An attractive hypothesis would be that intracellular carbohydrate levels are directly involved in regulation. Induction by carbohydrates has been demonstrated for the cytochrome *c* gene from sunflower (Felitti and Gonzalez 1998), and there are many examples of sugar-regulated genes in plants (Koch 1996, Jang and Sheen 1997). This would also explain the observed induction by light through photosynthetic activity (see below).

A further support for the existence of metabolic regulation of the cytochrome *c* gene is given by the fact that light promotes an increase in mRNA levels in the presence of acetate. In cells grown in minimal medium, a decrease in transcript levels is observed upon transfer to darkness, suggesting that the effect of light also occurs in the absence of acetate. The inhibitory effect of DCMU under both conditions suggests that an active photosynthetic electron transport chain is required for induction by illumination. It is likely that this is an indirect effect due to metabolic modifications undergone by cells upon illumination. It should be noted that, in a previous study, Amati et al. (1988) have not observed differences in cytochrome *c* mRNA levels in cell cultures grown in the presence of acetate either in complete darkness or under continuous illumination. We think this may be due to differences in growth conditions or to the fact that we have analyzed changes at short times (hours) after the illumination conditions were modified. After prolonged incubation in the presence of acetate, steady-state RNA levels tend to be similar either in darkness or under illumination, since the effect of light reaches a maximum level after 2–4 h of illumination and cells in darkness show a small but constant increase in transcript levels over time in the presence of acetate (Fig. 5A).

It is interesting to note that acetate represses the expression of some genes which products are directly involved in photosynthesis (Goldschmidt-Clermont 1986, Kindle 1987), while it behaves as an inducer of the cytochrome *c* gene. It would be interesting to know if other genes for respiratory components behave in a similar way. If so, a mechanism could be proposed by which the availability of a reduced carbon source would act to balance the expression of genes involved in carbon fixation and utilization. This reduced carbon source, in the form of acetate or, more likely, carbohydrates or triose phosphates, derived either from acetate incorporation or photosynthesis, would reflect the metabolic state of the cell and be the actual inducer. It is noteworthy that rapid growth of *Chlamydomonas* cultures requires both light and acetate, which have a rather additive effect. This additive effect is also observed on the induction of the cytochrome *c* gene. The availability of an energy source for growth would then be the metabolic signal involved in regulation. As mentioned above, carbohydrates are attractive candidates, since induction is intimately asociated with carbon assimilation. This constitutes our working hypothesis which will be tested by future experiments.

We have also analyzed the structure of the *Chlamydomonas* cytochrome *c* gene. This gene has a partially conserved intron/exon structure with respect to the corresponding genes from rice (Kemmerer et al. 1991) and *Arabidopsis thaliana*. This may indicate that two of the three introns were already present within the cytochrome *c* gene before the divergence of the lines leading to green algae and land plants. The third intron may be a more recent acquisition or may have been lost by the land plant lineage after its separation from the algal lineage. Introns have also been described in cytochrome *c* genes from animals and fungi (Scarpulla et al. 1981, Limbach and Wu 1983, Limbach and Wu 1985, Stuart et al. 1987), but none of them has a conserved position with respect to the *Chlamydomonas* gene.

The putative promoter region of the *Chlamydomonas* cytochrome *c* gene is composed of an 800-bp single copy region, preceded by a short repeated sequence present in several copies in the genome. Little is known about *cis*-acting sequences and *trans*-acting factors that operate in the expression of *Chlamydomonas* genes. So far, we have only noted the existence of sequences that match 70% with the tub box consensus (Brunke et al. 1984), and a heptamer recently proposed to be involved in light-regulated expression (Hahn and Kück 1999). We have not found significant similarities between upstream regions of the cytochrome *c* gene and other acetate-regulated genes. Functional and molecular studies will be necessary to elucidate which portions of the cytochrome *c* gene promoter are involved in the establishment of the observed expression pattern.

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