Differential Expression of the Arabidopsis Cytochrome *c* Genes *Cytc-1* and *Cytc-2*. Evidence for the Involvement of TCP-Domain Protein-Binding Elements in Anther- and Meristem-Specific Expression of the *Cytc-1* Gene[1]

Elina Welchen and Daniel H. Gonzalez*

Cátedra de Biología Celular y Molecular, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, 3000 Santa Fe, Argentina

The promoters of the Arabidopsis (*Arabidopsis thaliana*) cytochrome *c* genes, *Cytc-1* and *Cytc-2*, were analyzed using plants transformed with fusions to the β-glucuronidase coding sequence. Histochemical staining of plants indicated that the *Cytc-1* promoter directs preferential expression in root and shoot meristems and in anthers. In turn, plants transformed with the *Cytc-2* promoter fusions showed preferential expression in vascular tissues of cotyledons, leaves, roots, and hypocotyls, and also in anthers. Quantitative measurements in extracts prepared from different organs suggested that expression of *Cytc-1* is higher in flowers, while that of *Cytc-2* is higher in leaves. The analysis of a set of deletions and site-directed mutants of the *Cytc-1* promoter indicated that a segment located between −147 and −156 from the translation start site is required for expression and that site II elements (TGGGCC/T) located in this region, coupled with a downstream internal telomeric repeat (AAACCCTAA), are responsible for the expression pattern of this gene. Proteins present in cauliflower nuclear extracts, as well as a recombinant protein from the TCP-domain family, were able to specifically bind to the region required for expression. We propose that expression of the *Cytc-1* gene is linked to cell proliferation through the elements described above. The fact that closely located site II motifs are present in similar locations in several genes encoding proteins involved in cytochrome *c*-dependent respiration suggests that these elements may be the target of factors that coordinate the expression of nuclear genes encoding components of this part of the mitochondrial respiratory chain.

The respiratory chain of plant mitochondria is considerably more complex than the one found in animals. This is due to the presence of several NAD(P)H-dependent dehydrogenases and an alternative oxidase that drives electrons to oxygen directly from ubiquinone (Mackenzie and McIntosh, 1999). This additional complexity and the unique properties of plant energetic metabolism due to the existence of photosynthesis suggest that the expression of plant mitochondrial components may undergo specific regulatory mechanisms.

Mitochondrial biogenesis involves the expression of genes located in the nucleus and within the organelle. Several nuclear genes encoding mitochondrial components show increased expression in flowers (Huang et al., 1994; Felitti et al., 1997; Heiser et al., 1997; Zabaleta et al., 1998). This fits well with the fact that the number of mitochondria per cell increases considerably during flower development (Lee and Warmke, 1979). In situ hybridization studies have shown that the expression in flowers is cell-type specific and that there is a good correlation in the expression of several mitochondrial genes (Smart et al., 1994) and the nuclear gene encoding cytochrome *c* (Ribichich et al., 2001), suggesting the existence of coordinated regulatory mechanisms. Besides this organ- or cell-type-specific expression, we have observed that cytochrome *c* transcript levels are modified by incubation of plants in solutions containing metabolizable sugars or nitrogen salts (Felitti and Gonzalez, 1998; Welchen et al., 2002). This behavior was also reported for nuclear genes encoding cytochrome *c* oxidase subunits (Welchen et al., 2002, 2004; Curi et al., 2003).

To analyze the molecular mechanisms involved in the expression of plant respiratory chain components involved with cytochrome *c*-dependent respiration, we have characterized the promoter regions of the Arabidopsis (*Arabidopsis thaliana*) *Cytc-1* (At1g22840) and *Cytc-2* (At4g10040) genes encoding cytochrome *c*. We have determined that these genes show differential expression patterns and that a segment containing two site II (TGGGCC/T) elements, which interact with a TCP-domain transcription factor, and a downstream internal telomeric repeat are required for expression of the *Cytc-1* gene. Closely located site II motifs, which have been implicated in the expression of genes in proliferating cells (Kosugi et al., 1995; Kosugi and Ohashi, 1997; Trémousaygue et al., 2003), are present in the promoter regions of other genes encoding components of the cytochrome *c*-dependent respiratory pathway,

[1] This work was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica, Fundación Antorchas, and Universidad Nacional del Litoral. D.H.G. is a member of CONICET; E.W. is a fellow of the same institution.

* Corresponding author; e-mail dhgonza@fbcb.unl.edu.ar; fax 54–342–4575219.

Article, publication date, and citation information can be found at www.plantphysiol.org/cgi/doi/10.1104/pp.105.065920.

suggesting the existence of a link between cell proliferation and the biogenesis of this part of the mitochondrial electron transport chain.

RESULTS

The *Cytc-1* Promoter Directs Tissue-Specific β-Glucuronidase Expression

To characterize the expression patterns conferred by the promoter region of the Arabidopsis *Cytc-1* gene, we cloned a 1,236-bp genomic fragment covering nucleotides −1,182 to +54 with respect to the ATG start codon in frame with the β-glucuronidase (*gus*) gene-coding region contained in the binary vector pBI101.3. This clone was used to obtain Arabidopsis transgenic plants by Agrobacterium-mediated transformation. Several independent transgenic lines were analyzed by histochemical staining of GUS activity using 5-bromo-4-chloro-3-indolyl-β-D-GlcUA (X-gluc) as substrate. Three-day-old seedlings grown on petri dishes on Murashige and Skoog medium showed staining only in the root meristem (Fig. 1A). Upon progression of development, GUS activity was detected in the shoot apical meristem, at the tip of cotyledons, and in nascent leaf primordia (Fig. 1, B–E). Specific expression was also observed at the root tips, in nascent secondary roots, and at the junction of secondary and primary roots (Fig. 1, G, H, and L). At the reproductive stage, expression was observed only in anthers, especially in pollen grains, and in stigmatic papillae (Fig. 1, J and K). No activity could be detected in hypocotyls, stems, leaves, siliques, or other flower organs (Fig. 1, A, F, I, and K), suggesting that the *Cytc-1* gene promoter directs preferential expression in specific cell types. Mascarenhas and Hamilton (1992) reported the existence of artifactual staining of pollen due to the diffusion of dye produced in other parts of the anther. This produces variable staining of pollen grains even in homozygous plants. The uniform staining pattern observed in plants transformed with the *Cytc-1* promoter construct suggests that this is not an artifact of the type mentioned above.

Expression of the *Cytc-2* Gene Differs from That of the *Cytc-1* Gene

The promoter region covering nucleotides −967 to +54 of the Arabidopsis *Cytc-2* gene was also analyzed using *gus* fusions and plant transformation. Analysis of transformed plants indicated that the *Cytc-2* promoter directs a much broader expression pattern. GUS activity was observed in vascular tissues of roots, hypocotyls, cotyledons, and leaves (Fig. 1, M–R). No activity was detected in meristems or nascent leaves or secondary roots (Fig. 1, O, S, and T). In reproductive organs, expression was evident in developing anthers and pollen, in petal veins, and at the junction of flowers and siliques with pedicels (Fig. 1W). Expression was also detected within siliques, specifically in the septum and the funiculus (Fig. 1, U and V). *Cytc-2* promoter activity differs, then, from that observed with the *Cytc-1* promoter, showing overlap only in anthers.

The amount of GUS activity present in protein extracts from different organs of plants transformed with either the *Cytc-1* or the *Cytc-2* promoter fusions was quantitatively assessed by a fluorometric GUS assay (Fig. 2). Specific GUS activity in seedlings (data not shown) and rosette leaves was not significantly different from basal levels (i.e. those obtained with plants transformed with the promoterless *gus* gene present in pBI101.3) in plants transformed with *Cytc-1*. For this construct, activity was highest in flowers and was also detected in extracts from roots and siliques (Fig. 2). For *Cytc-2*, highest expression was observed in leaves, and activities in flowers and siliques were also higher than those observed with the *Cytc-1* promoter (Fig. 2). These results agree with those obtained using histochemical detection of GUS activity.

Deletion Analysis of the *Cytc-1* Promoter

To define the minimal promoter regions required for correct *Cytc-1* gene expression, we performed a series of nested deletions from the upstream portion of the 1,234-bp fragment fused to *gus* (Fig. 3A). The different constructs were introduced into Arabidopsis and GUS activity was analyzed in transformed plants. Histochemical assays indicated that a fragment located between −147 and −218 of the start codon is absolutely required for *Cytc-1* transcription, since no GUS activity could be detected in any tissue or cell type of plants carrying the proximal 200-bp fragment (−146 to +54) fused to *gus* (data not shown). Fluorometric assays using extracts from different organs were in agreement with histochemical assays, since GUS activity values decreased to those observed with the promoterless *gus* gene (Fig. 3B). A smaller deletion, down to nucleotide −218, produced plants with expression in flowers reduced to about 50%, suggesting that a positive element is present between −219 and −369 (Fig. 3B).

Deletion of the segment located between −147 and −218 within the context of the −369 promoter fragment completely abolished expression in all organs, indicating that upstream sequences cannot functionally replace this region (Fig. 3B). The inclusion of the essential fragment in front of a −90 cauliflower mosaic virus 35S minimal promoter was unable to confer specific GUS expression to transformed plants (data not shown). Accordingly, additional sequences located downstream of −147 also seem to be required for expression. Otherwise, since the *Cytc-1* gene contains a TATA-less promoter, it may be that this segment does not function correctly within the context of the cauliflower mosaic virus 35S promoter that contains a TATA box.

Mutagenesis of the Promoter Segment Required for *Cytc-1* Gene Expression

A set of 10-bp scanning mutations along the 72-bp (−147/−218) fragment required for expression of the

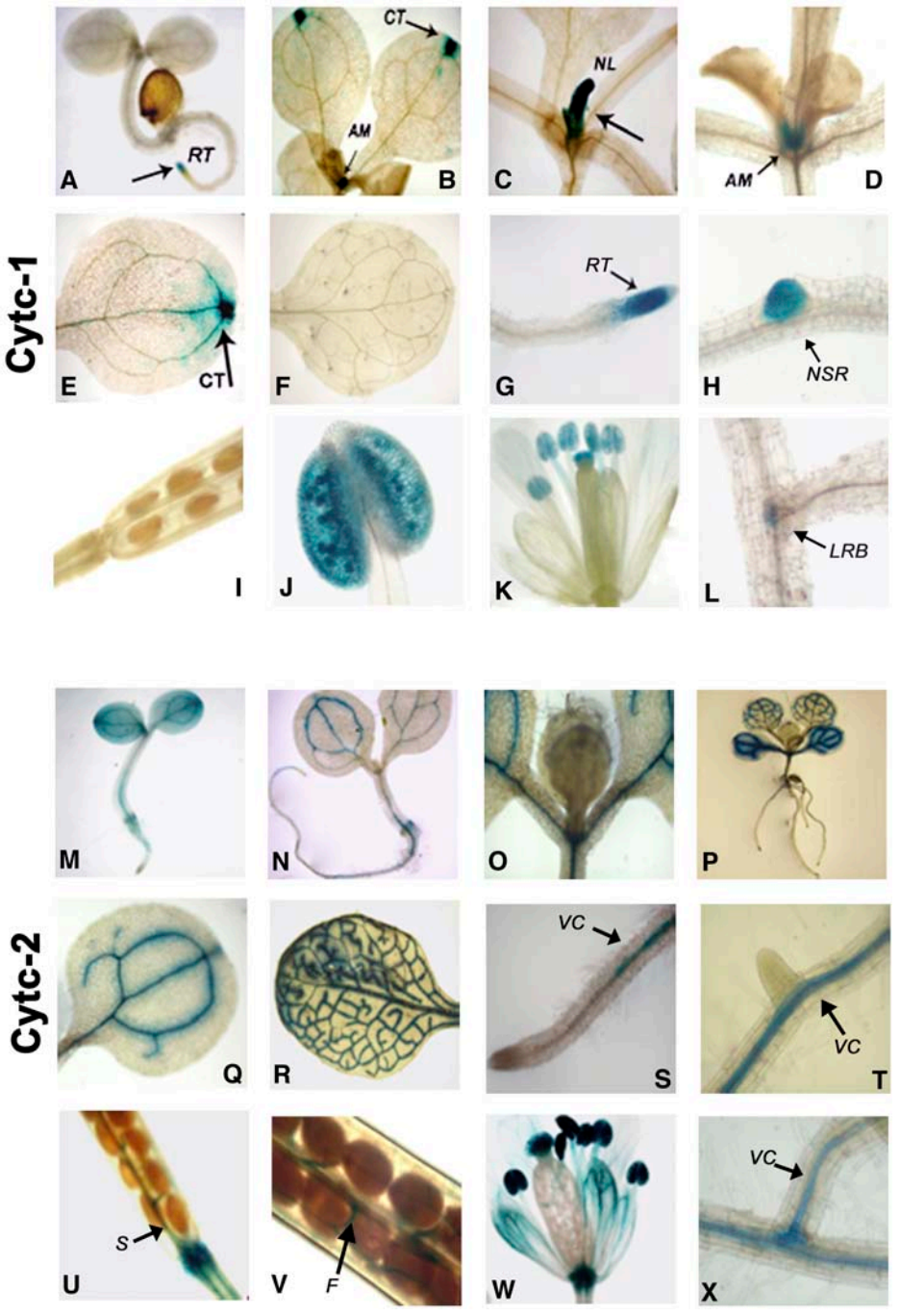

Figure 1. Histochemical localization of GUS activity in Arabidopsis plants transformed with either the *Cytc-1* (A–L) or *Cytc-2* (M–X) promoters fused to the *gus* reporter gene. A, Three-day-old seedling showing staining in the root tip (RT). B, Upper portion of a 7-d-old seedling showing staining in cotyledon tips (CT) and apical meristem (AM). C and D, Upper portion of 12- and 15-d-old seedlings showing staining in nascent leaves (NL) and apical meristem (AM). E and F, Cotyledon and leaf of a 20-d-old plant. G and H, Primary root showing staining at the tip (RT) and nascent secondary root (NSR). I, Silique. J, Anther. K, Flower. L, Lateral root showing staining at the base (LRB). M to P, Seedlings at different developmental stages. Q and R, Cotyledon and leaf of a 20-d-old plant. S and T, Primary root and nascent secondary root showing staining in the vascular cylinder (VC). U and V, Siliques showing staining in the septum (S) and the funiculus (F). W, Flower. X, Lateral root.

Cytc-1 gene was used to map the presence of discrete regulatory elements (Fig. 4A). Fluorometric assays using extracts from different organs of plants transformed with the mutagenized 272-bp proximal promoter fragments (−218 to +54) fused to *gus* revealed that mutation of positions −147 to −156 produced very low GUS activity levels, similar to those observed with the promoterless *gus* gene (Fig. 4B). In addition, mutation of the adjacent 10-bp segment (−157 to −166) produced a moderate decrease in expression (Fig. 4B). Plants transformed with the mutagenized −147 to −156 fragment did not show GUS histochemical staining in any tissue analyzed (Fig. 5, D, J, P, and V). All other mutants showed GUS activity in anthers and meristems with a similar location to that observed in plants transformed with the nonmutagenized fragment (Fig. 5, F, L, R, and X), but in plants carrying mutations from −157 to −166 the intensity of staining was reduced with respect to plants containing the wild-type promoter fragment (data not shown).

The 10-bp promoter segment required for expression contains a copy of the so-called site II element (Fig. 4A),

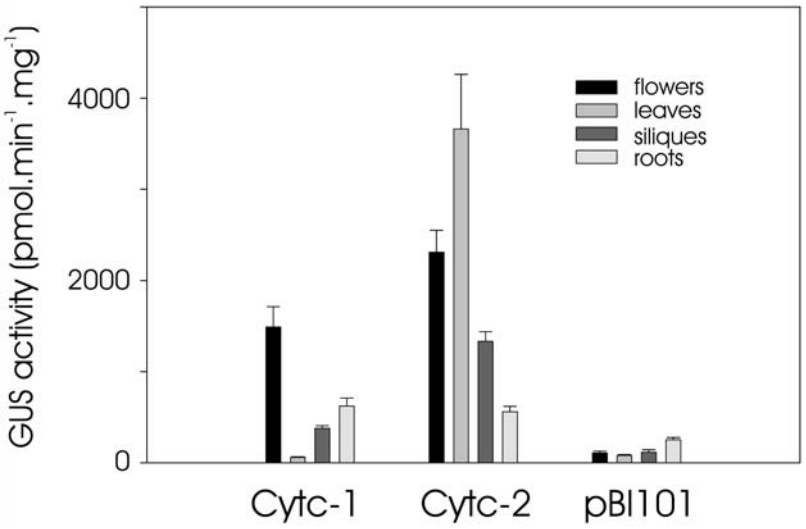

Figure 2. Analysis of GUS activity in protein extracts from different organs of plants transformed with either the *Cytc-1* or the *Cytc-2* promoter-*gus* fusions. Specific GUS activity was determined using protein extracts prepared from different organs (as indicated) of plants carrying either the *Cytc-1* or the *Cytc-2* promoters fused to *gus*. Activity was also measured in extracts from plants transformed with a promoterless *gus* gene (pBI101). Bars indicate the mean activity values obtained with organs from three independent transformants for each construct. Error bars represent SE. Similar results were obtained in other experiments in which different lines were used.

which has been implicated in the expression of genes in proliferating cells (Kosugi et al., 1995; Kosugi and Ohashi, 1997; Trémousaygue et al., 2003). This element has the consensus sequence TGGGCC/T and is usually present in more than one copy located proximal to each other and within −120 to −180 of the start codon (Trémousaygue et al., 2003). Indeed, a second site II element is present in the *Cytc-1* gene promoter between −166 and −171 (Fig. 4A). To analyze the function of site II elements in the expression of the *Cytc-1* gene, we have

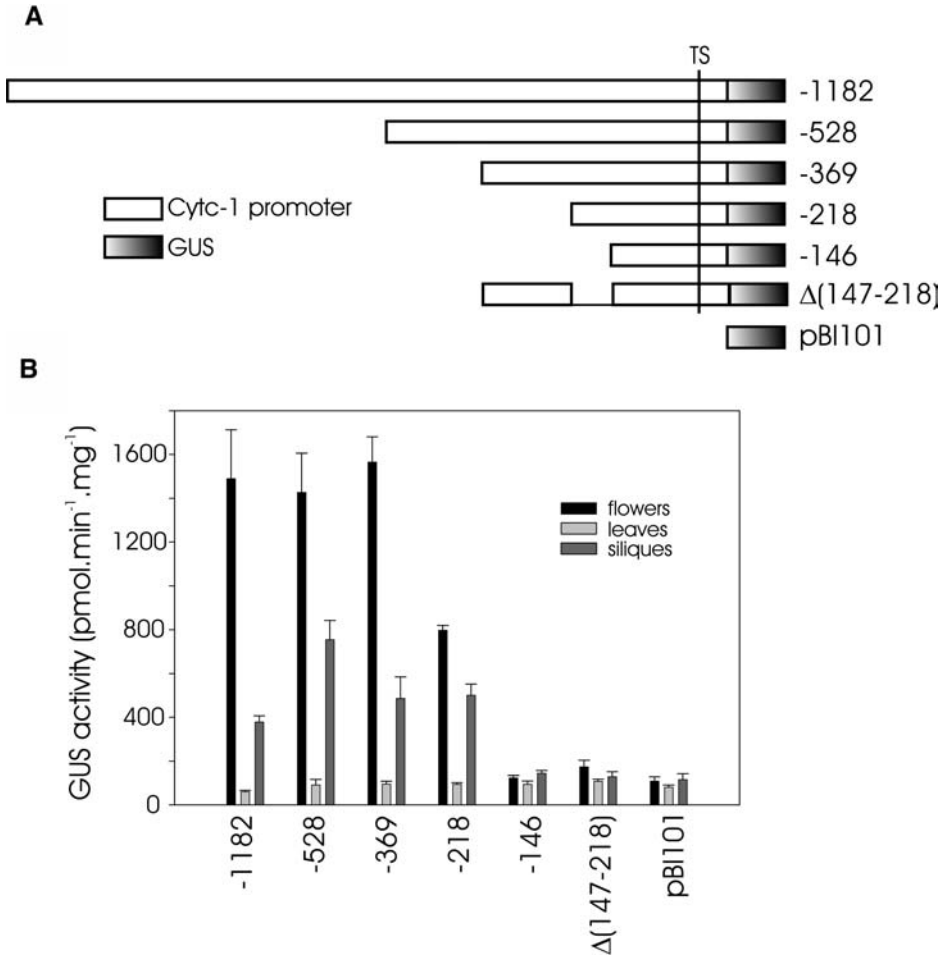

Figure 3. Analysis of GUS activity driven by truncated forms of the *Cytc-1* promoter. A, Constructs used for the analysis of the *Cytc-1* promoter. Numbers indicate the upstream end of the promoter fragment present in each construct, with respect to the translation start site (TS); the downstream end was at +54 for all constructs. Construct Δ (147–218) carries a deletion comprising the indicated nucleotides within the context of the −369 promoter fragment. Plants transformed with the promoterless *gus* gene (pBI101) were also used. B, GUS expression levels in organs of plants carrying the different constructs. The results indicate the mean (± SE) of three independent lines. Numbers below the bars indicate the respective construct. Similar results were obtained in other experiments in which different lines were used.

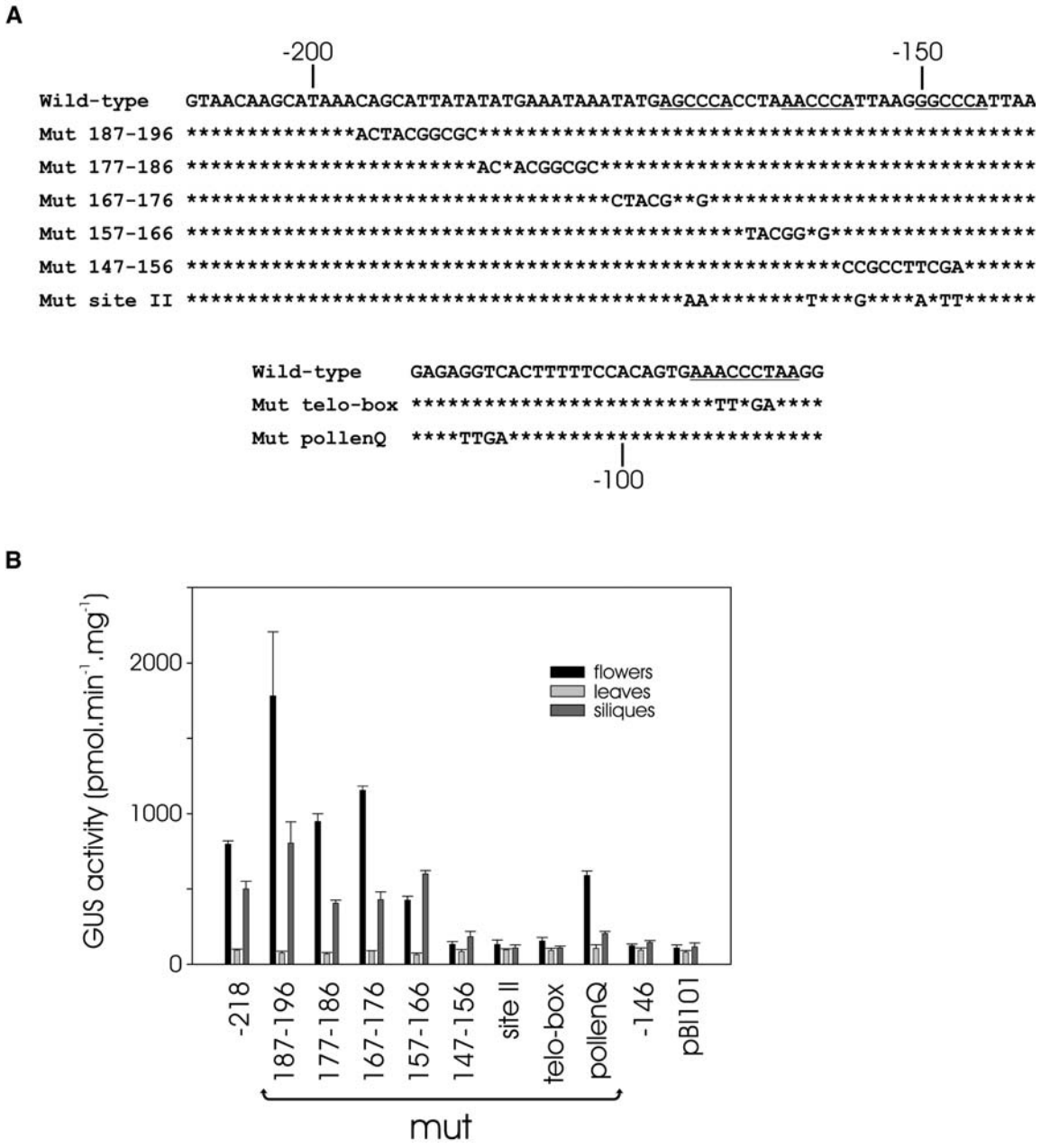

Figure 4. Mutagenic analysis of the *Cytc-1* promoter region required for expression. A, Sequence of the *Cytc-1* promoter regions that were analyzed by mutagenesis. Introduced mutations are specified below the wild-type sequence; asterisks indicate identical nucleotides. Numbers indicate the position relative to the translation start site. Site II elements and the *telo*-box are underlined. B, Specific GUS activity was determined using protein extracts prepared from different organs (as indicated) of plants carrying mutagenized promoter fragments fused to *gus*. All constructs contain the region-spanning nucleotides −218 to +54 of the *Cytc-1* gene. Activity was also measured in extracts from plants transformed with the −146 to +54 fragment (−146) and with the promoterless *gus* gene (pBI101). The bars represent mean (± SE) values for three independent lines carrying each construct. Similar results were obtained in other experiments in which different lines were used.

constructed a mutant promoter in which both elements were mutagenized by changing the two central nucleotides (GG) to either A or T (Fig. 4A). A similar sequence located between the site II elements (TGGGTT) was also mutagenized to TGGATT in this construct. Histochemical staining of plants transformed with the mutagenized promoter indicated that the introduced mutations caused a complete loss of expression in all organs and developmental stages (Fig. 5, C, I, O, and U). Fluorometric assays revealed the presence of basal levels of GUS activity similar to those found in plants transformed with the promoterless *gus* gene (Fig. 4B). The results suggest that the integrity of site II elements is essential for *Cytc-1* gene expression.

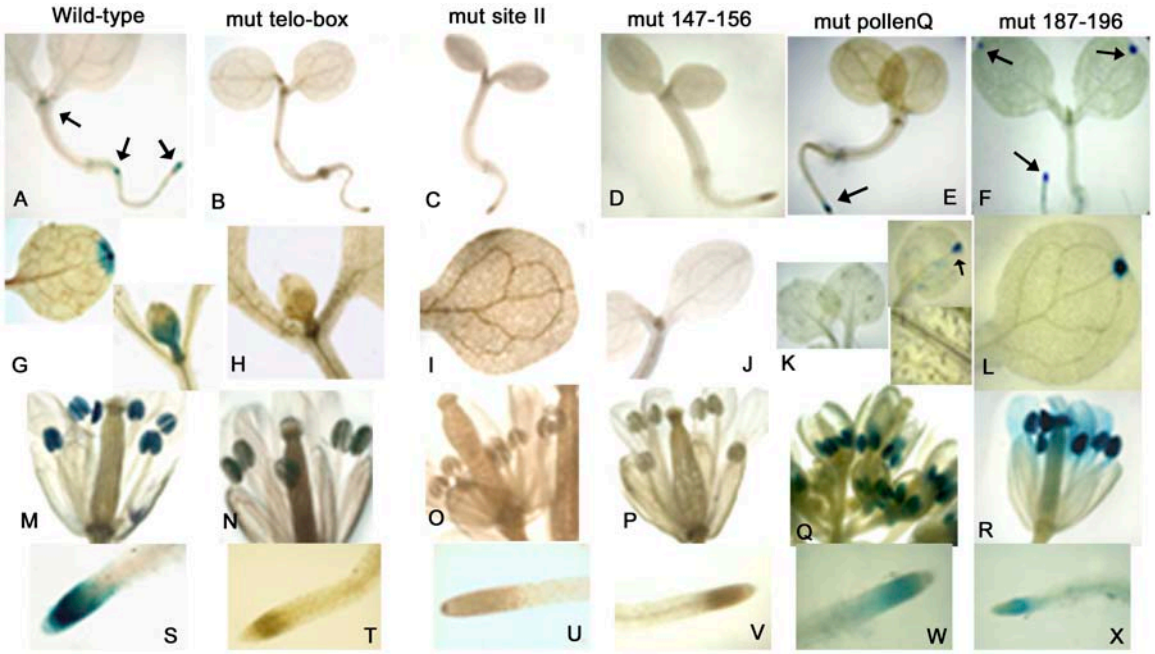

Figure 5. Histochemical analysis of GUS activity in Arabidopsis plants transformed with mutagenized promoters fused to the *gus* reporter gene. Seedlings (A–F), cotyledons and nascent leaves (G–L), flowers (M–R), or roots (S–X) from plants transformed with either the wild-type (−218) promoter or the same promoter with mutations (as described in Fig. 4) in the *telo*-box, the site II elements, the pollenQ motif, or the regions from −147 to −156 or from −187 to −196 were analyzed by histochemical staining.

Site II elements are usually present together with downstream internal telomeric repeats or *telo*-boxes (AAACCCTAA; Trémousaygue et al., 2003). A sequence perfectly matching the *telo*-box is located between −86 and −94 in the *Cytc-1* gene (Fig. 4A). Plants bearing a construct in which the *telo*-box was modified displayed very low GUS activity in anthers when analyzed by the histochemical method (Fig. 5N), but no staining could be observed in other parts of the plant (Fig. 5, B, H, and T). Activity in anthers was so low that it could not be differentiated from the activity present in plants transformed with the promoterless *gus* gene when extracts from whole flowers were analyzed by the fluorometric method (Fig. 4B). It is then likely that this mutation causes a general decrease in expression, so that GUS activity becomes detectable only in anthers, where expression levels driven by the wild-type promoter are higher. These results agree with the fact that mutation of the *telo*-box in other genes does not cause a complete loss of expression, suggesting that this element acts as a stimulatory element coupled to site II or other motifs (Trémousaygue et al., 1999, 2003; Manevski et al., 2000).

Finally, a region containing the sequence AGGTCA, described as a quantitative element required for high-level expression in pollen of the ZM13 maize pollen-specific gene (Hamilton et al., 1998), was identified between −109 and −114 (Fig. 4A, pollenQ). Considering that the *Cytc-1* gene shows expression in pollen, the relevance of this element for expression was also studied by mutagenesis. Plants with the mutagenized *Cytc-1* promoter fused to *gus* showed lower specific activity values (Fig. 4B), suggesting that the presence of the AGGTCA element may cause a moderate increase in *Cytc-1* gene expression levels. Histochemical staining of these plants did not reveal any difference, however, with plants bearing the wild-type promoter fragment (Fig. 5, E, K, Q, and W).

Induction of the *Cytc-1* Promoter by Sucrose and the Cytokinin 6-Benzylaminopurine

We have previously shown that the *Cytc-1* gene is transcriptionally regulated by carbohydrates (Welchen et al., 2002). GUS activity measurements in extracts from seedlings carrying different promoter deletions indicated that all constructs that produced GUS expression also showed induction by carbohydrates at similar levels than those observed with the larger construct (data not shown). This was also true for plants carrying mutagenized promoter fragments, except for the ones with changes between nucleotides −157 to −166, in which very low induction levels were observed (Fig. 6). Since these plants also show lower GUS activity in other organs (e.g. flowers), it can be speculated that the putative elements involved in induction by carbohydrates may be the same as those required for increasing the expression in organs like flowers.

The fact that the *Cytc-1* gene shows preferential expression in proliferating tissues led us to investigate the effect of the inclusion of cytokinins in the culture medium on GUS activity levels driven by different *Cytc-1*

promoter fragments. The results obtained indicated that *Cytc-1* expression in seedlings is induced by treatment with the cytokinin 6-benzylaminopurine (BAP; Fig. 6). The response of the different constructs closely matched the one observed for Suc treatments, but activity values were higher in the presence of the hormone. These observations reinforce the notion that expression of the *Cytc-1* gene is governed by elements that respond to cell proliferation.

Proteins Present in Cauliflower Nuclear Extracts Specifically Bind to the *Cytc-1* Promoter

The presence of nuclear proteins that bind to the *Cytc-1* promoter region required for expression was investigated using extracts prepared from cauliflower (*Brassica oleracea*) inflorescences. Cauliflower was chosen because it is a readily available source of proteins expressed in meristematic tissues and because of its proximity to Arabidopsis. Figure 7A (lanes 1 to 3) shows a mobility shift assay using the inflorescence nuclear extract and fragments comprising either nucleotides −21 to −218, −21 to −146, or −126 to −218, respectively. It can be observed that both fragments that contain the region required for expression produce several shifted complexes in a similar pattern (Fig. 7A, lanes 1 and 3), while only a faint shifted band was obtained when the region between −147 and −218 was removed (Fig. 7A, lane 2). This result suggests that the cauliflower nuclear extract contains proteins that specifically bind to this region of the *Cytc-1* promoter. Indeed, when a labeled fragment comprising nucleotides −126 to −218 mutagenized in its site II elements was used, no binding was observed, indicating that the nuclear proteins bind to the same region that is essential for *Cytc-1* expression (Fig. 7A, lane 4). A similar conclusion could be drawn from an experiment in which excess unlabeled wild-type and mutant fragments were used to compete binding to labeled DNA (Fig. 7B). While either a 10- or a 50-fold molar excess of the wild-type fragment produced a strong decrease in binding, almost no competition was observed when similar amounts of the mutant fragment were used (Fig. 7B, lanes 1 to 5). The small amount of binding observed with the mutant fragment was also more effectively competed by DNA containing nonmutated site II elements (Fig. 7B, lanes 6 to 10). We speculate that proteins with the same binding specificity present in Arabidopsis act as key regulators of *Cytc-1* gene expression.

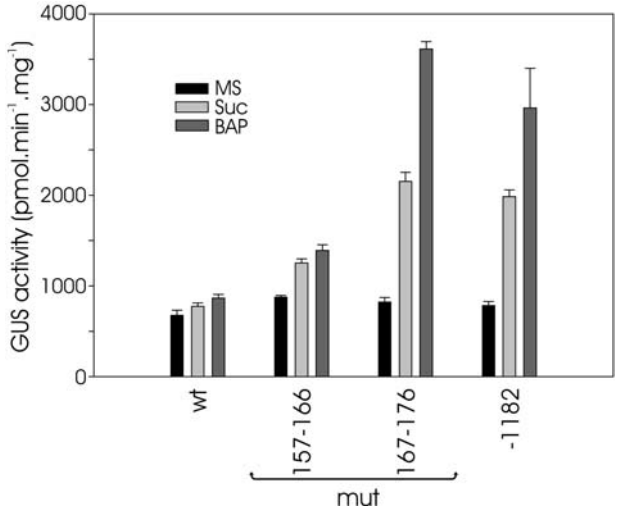

Figure 6. Induction of the *Cytc-1* gene by Suc and cytokinins. GUS activity was measured using the fluorogenic substrate MUG and protein extracts prepared from 7-d-old seedlings grown in Murashige and Skoog (MS) medium alone or supplemented with either 3% Suc or 50 μM BAP, as indicated. The plants used for the analysis were a mix of four independent lines for each construct, as indicated. Activity was also measured in extracts from nontransformed plants (wt). Similar results were obtained in other experiments in which different lines were used.

A TCP-Domain Protein Binds to the *Cytc-1* Promoter Region Required for Expression

It has been proposed that site II elements are binding sites for a group of transcription factors that contain the so-called TCP domain (Kosugi and Ohashi, 1997; Trémousaygue et al., 2003). The ability of the relevant region of the *Cytc-1* promoter to function as a TCP-domain protein-binding site was assayed in vitro, using recombinant protein AtTCP20 (Trémousaygue et al., 2003) in electrophoretic mobility shift assays (Fig. 7C). Specific retarded bands were observed when this protein was incubated with DNA representing promoter regions from −126 to −218, even in the presence of an excess of unspecific competitor (Fig. 7C, lane 1). Mutations spanning nucleotides −187 to −196 or −177 to −186 did not affect binding (Fig. 7C, lanes 2 and 3). Conversely, when a fragment with mutagenized site II elements was used, no binding was observed (Fig. 7C, lane 4). These results suggest that members of the TCP family are likely candidates to act as regulators of *Cytc-1* gene expression.

In a similar way, we also tested the ability of the region of the *Cytc-1* promoter containing the *telo*-box to bind recombinant protein AtPuralpha, which has been shown to interact with these elements in vitro (Trémousaygue et al., 1999). Electrophoretic mobility shift assays with wild-type fragments covering nucleotides −21 to −218 or −21 to −146 showed a specific retarded band that was not observed when a promoter fragment with mutations within the *telo*-box was used (Fig. 7D). These results indicate the existence of specific binding of AtPuralpha to the *telo*-box present in the *Cytc-1* promoter.

In support of a functional role of site II elements, we have also noted the presence of similar motifs in cytochrome *c* genes from other plants. Searches in data banks revealed the existence of four sequenced genomic regions located upstream of cytochrome *c*-encoding sequences: two from rice (*Oryza sativa*) and one each from *Brassica rapa* and *Medicago truncatula*. The sequence from *B. rapa* (accession no. AC155340), more

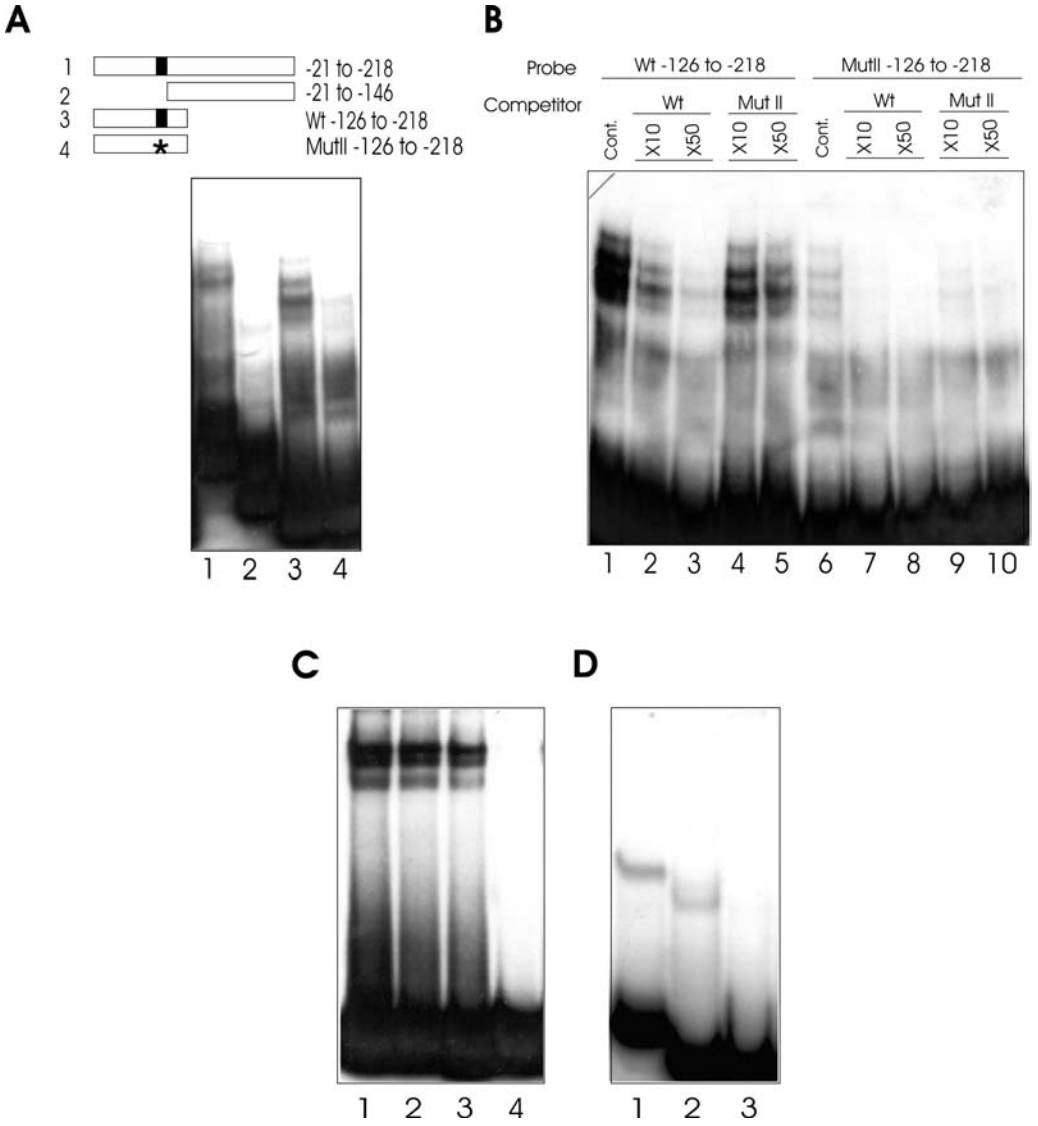

Figure 7. Nuclear proteins from cauliflower inflorescences and recombinant proteins AtTCP20 and AtPuralpha specifically bind to the *Cytc-1* promoter. A, Nuclear extracts (3 μg) from cauliflower inflorescences were analyzed by an electrophoretic mobility shift assay for the presence of proteins that bind to labeled DNA spanning nucleotides −21 to −218 (lane 1), −21 to −146 (lane 2), or −126 to −218 (lane 3). In lane 4, a fragment spanning nucleotides −126 to −218 with mutations in its site II elements (as described in Fig. 4) was used. B, The binding of nuclear proteins to labeled fragment −126 to −218 was analyzed in the presence of only unspecific competitor (lane 1) or with the addition of a 10- or a 50-fold molar excess of unlabeled wild-type (lanes 2 and 3) or mutated forms (lanes 4 and 5) of the same fragment. In lanes 6 to 10, a similar experiment was carried out using the mutated form of the fragment as labeled probe. C, Binding of recombinant protein AtTCP20 to the *Cytc-1* promoter. An aliquot (200 ng) of recombinant protein AtTCP20 was analyzed by an electrophoretic mobility shift assay for binding to a *Cytc-1* promoter fragment spanning nucleotides −126 to −218 (lane 1) or to the same fragment with mutations introduced in the regions −187/196 (lane 2), −177/−186 (lane 3), or in the site II elements (lane 4). D, Binding of recombinant protein AtPuralpha (200 ng) to *Cytc-1* promoter fragments spanning nucleotides −21 to −218 (lane 1) or −21 to −146 (lane 2), or to a similar fragment with mutations within the *telo*-box (lane 3) as described in Figure 4.

closely related to Arabidopsis, contains four site II motifs located at −359, −250, −160, and −133 from the translation start site. In Medicago (accession no. AC142095), three motifs are present at −925, −908, and −136, respectively. From the two rice genes, the one located in chromosome 5 (AC137623), which has been shown to be expressed at high levels and in several parts of the plant (Jang et al., 2002), contains site II motifs at −295 and −81, while the gene from chromosome 1 (AP003379) has site II motifs at −641, −211, −93, and −84. It is noteworthy that this gene also contains a downstream *telo*-box at −27, thus presenting an arrangement very similar to the one observed in the Arabidopsis *Cytc-1* gene. A *telo*-box is also present in the Brassica gene, but located upstream of the site II motifs in this case (−381).

Several Nuclear Genes Involved in Cytochrome *c*-Dependent Respiration Contain Site II Motifs in Their Promoters

The relevance of site II elements for *Cytc-1* gene expression led us to investigate the existence of similar motifs in genes encoding other components of cytochrome *c*-dependent respiration, assuming that the expression of these components must be somehow coordinated. We have analyzed 17 Arabidopsis nuclear genes encoding either cytochrome *c* oxidase subunits or proteins putatively involved in the biogenesis of this complex. Two or more neighboring site II motifs, located around −200 of the translation start site, could be identified in 11 of them, representing 65% of examined promoters (Table I). A similar arrangement was observed in 9 out of 15 genes (60%) encoding complex III subunits. This is considerably more frequent than expected by chance. Indeed, when a similar search was conducted using the −1 to −500 region of 93 randomly selected Arabidopsis genes, only in 17% of them could neighboring site II motifs, separated by 25 bp or less, be detected. These results strongly suggest that site II motifs may be targets of factors that regulate the coordinated expression of nuclear genes encoding components of this portion of the plant respiratory chain. The relevance, if any, of these elements must be assessed by mutagenic analysis of the respective promoter fragments.

Table I. *Site II motifs present in promoters of Cytc, COX, and complex III genes*

Gene	MIPS Code	No. of Site II Elements[a]	Location[b]
Cytc	At1g22840	2	−147/−168
	At4g10040	2	−259/−271
COX5b	At1g80230	1	−145
	At3g15640	–	–
COX5c	At2g47380	3	−151/−170/−383[c]
	At3g62400	2	−128/−141[c]
	At5g61310	–	–
COX6a	At4g37830	3	−116/−127/−138
COX6b	At1g22450	2	−190/−217
	At4g28060	2	−157/−173
	At5g57815	3	−128/−140/−151
COX10	At2g44520	2	−210/−267
COX11	At1g02410	3	−294/−308/−318[c]
COX15	At5g56090	–	–
COX17	At1g53030	4	−120/−131/−142/−453[c]
	At3g15352	3	−206/−228/−235[c]
COX19	At1g66590	2	−154/−164
	At1g69750	2	−154/−164
SCO1	At3g08950	1	−232
CYC1	At3g27240	3	−169/−179/−201[c]
	At5g40810	2	−199/−222[c]
MPPalpha	At1g51980	3	−179/−220/−227
	At3g16480	3	−172/−195/−231
MPPbeta	At3g02090	2	−266/−278
QCR6	At1g15120	3	−156/−183/−228[c]
	At2g01090	2	−147/−201[c]
QCR7	At4g32470	4	−133/−145/−206/−241
	At5g25450	–	–
QCR9	At3g52730	4	−164/−188/−230/−304
UCR1	At5g13430	–	–
	At5g13440	1	−314
UCRQ	At3g10860	–	–
	At5g05370	2	−129/−147
UCRY	At2g40765	–	–

[a]Number of site II motifs between −1 and −500 from the translation start site. [b]Corresponding to the translation start site. [c]These genes contain introns in the 5′-noncoding region. The location of the site II motifs is referred to as the translation start site, not considering intron sequences.

DISCUSSION

Little is known about the molecular mechanisms involved in the expression of plant mitochondrial components. It has been shown that several nuclear genes encoding mitochondrial polypeptides are preferentially expressed in flowers (Huang et al., 1994; Felitti et al., 1997; Heiser et al., 1997). Studies using in situ hybridization and promoter/*gus* fusions have shown that localized expression in anthers seems to be a common feature of most genes encoding respiratory chain components (Zabaleta et al., 1998; Ribichich et al., 2001; Elorza et al., 2004; Welchen et al., 2004). Expression in other parts of the plant, however, is different depending on the particular gene analyzed.

In this article, we have studied the expression patterns conferred by sequences located upstream of both Arabidopsis genes encoding cytochrome *c*. We have decided to analyze fragments from approximately 1 kb, assuming that most elements relevant for expression would be located within this range. For the *Cytc-1* gene, the fragment used comprises the entire intergenic region plus coding sequences of another gene (At1g22830).

Both upstream fragments direct the expression of a reporter gene in anthers, but different expression patterns were observed in other parts of the plant. *Cytc-1* promoter activity is highly localized to root and shoot apical meristems, while the *Cytc-2* gene promoter is active in cotyledons and vascular tissues of roots, hypocotyls, and leaves. It seems, then, that both promoters have divergently evolved to meet expression requirements in different parts of the plant. Differential expression patterns were also observed for genes encoding the alternative oxidase from soybean (Thirkettle-Watts et al., 2003) and the complex II iron-sulfur subunit from Arabidopsis (Elorza et al., 2004). Common expression in anthers, on the other hand, may be related to the importance of mitochondrial function for pollen development (Hanson, 1991). It is also noteworthy that GUS expression is not observed for any of the *Cytc* genes in several tissues. This fact does not necessarily mean that *Cytc* genes are not expressed at all in these tissues, but rather that expression levels are too low to be detected. Assuming that cytochrome *c*-dependent respiration is probably necessary in most cell types, our results point to the existence of tissues

Table II. *List of oligonucleotides used for promoter constructs*

Primer Name	Sequence (5′→3′)	Position[a]	Gene	Sense[b]	Cloning Site
CATP11	GGCAAGCTTCATTAAGATTGGAGATAC	−146	*Cytc-1*	Direct	*Hin*dIII
CATP12	GCAGAATTCGCAGTATCTCCAATCTTA	−126	*Cytc-1*	Reverse	*Eco*RI
CATP13	GGCAAGCTTGTAGAAGTAGGATAAAGA	−369	*Cytc-1*	Direct	*Hin*dIII
CATP15	GAGCAAGCTTGTAACGGT	−532	*Cytc-1*	Direct	*Hin*dIII
CATP16	CCTGAAGATCTTCTCACC	+60	*Cytc-1*	Reverse	*Bgl*II
CATP17	AAGTATGAGTAACAAGCAT	−218	*Cytc-1*	Direct	–
CATP19	GCAAGCTTATATACAACAAGTTTT	−293	*Cytc-1*	Direct	–
delA1	ATCTTAATGGCGAAGGACCATCCCCCTA	−138	*Cytc-1*	Reverse	–
delA2	GTCCTTCGCCATTAAGATTGGAGATAC	−227	*Cytc-1*	Direct	–
mutA1	ACTACGGCGCATTAAGGGCCCATTAAGA	−166	*Cytc-1*	Direct	–
mutA2	GCGCCGTAGTGGGCTCATATTTATTTCA	−157	*Cytc-1*	Reverse	–
mutA3	ACTACGGCGCACCTAAACCCATTAAGGG	−176	*Cytc-1*	Direct	–
mutA4	GCGCCGTAGTTTATTTCATATATAATGCTG	−167	*Cytc-1*	Reverse	–
mutA5	ACTACGGCGCATATGAGCCCACCTAAAC	−186	*Cytc-1*	Direct	–
mutA6	GCGCCGTAGTTATAATGCTGTTTATGCTT	−177	*Cytc-1*	Reverse	–
mutA7	ACTACGGCGCTATGAAATAAATATGAGC	−196	*Cytc-1*	Direct	–
mutA8	GCGCCGTAGTTTTATGCTTGTTACTCATA	−187	*Cytc-1*	Reverse	–
mutA9	CCGCCTTCGACATTAAGATTGGAGATAC	−156	*Cytc-1*	Direct	–
mutA10	TCGAAGGCGGGGGTTTAGGTGGGCTCAT	−147	*Cytc-1*	Reverse	–
mutII-1	ACCTAAATCCAGTAAGAGTTCATTAAGATTG	−166	*Cytc-1*	Direct	–
mutII-2	TCTTACTGGATTTAGGTGTTCTCATATTTATTTC	−150	*Cytc-1*	Reverse	–
TELOmut-1	GTGAATTCGAAAGGAAACTTCGGCAAGC	−97	*Cytc-1*	Direct	–
TELOmut-2	TCCTTTCGAATTCACTGTGGAAAAAGTG	−83	*Cytc-1*	Reverse	–
mutQ-1	TTTTTAAGAGATTGAACTTTTTCCAC	−124	*Cytc-1*	Direct	–
mutQ-2	GTGGAAAAAGTTCAATCTCTTAAAAA	−99	*Cytc-1*	Reverse	–
CATP22	GCACTTGGTTCTGAAGAT	+69	*Cytc-2*	Reverse	–
CATP26	CCGAAGCTTTAGGAGAAAAATGGTATT	−967	*Cytc-2*	Direct	*Hin*dIII

[a]Position of the 5′-end with respect to the translation start site. [b]Corresponding to the coding strand.

where higher expression levels are produced, probably reflecting higher demands for this metabolism.

The expression patterns observed with the *Cytc-1* promoter closely match the distribution of transcripts determined by in situ hybridization studies in both sunflower and Arabidopsis (Ribichich et al., 2001). In that study, transcripts were detected mainly in anthers and root meristems, but no specific hybridization was evident in mature leaves, although expression in these organs was observed in northern experiments (Felitti et al., 1997; Welchen et al., 2002). One can speculate that the northern results with leaf RNA represent low-level expression in all leaf cell types, thus explaining the lack of signal in in situ hybridization (Ribichich et al., 2001) and GUS histochemical analysis (this study). However, this low-level expression was not even detected in GUS activity measurements using leaf protein extracts. This may arise from the fact that all *Cytc-1* promoter constructs used in this study produce rather low GUS activity values, thus obscuring the detection of low expression levels, since they become not significantly different from background activity levels. In agreement with our results, GUS activity was not detected in mature leaf tissues when using promoters from nuclear genes encoding a complex II subunit, although the presence of endogenous transcripts could be detected (Elorza et al., 2004).

Previous northern analysis of *Cytc-1* expression in different organs suggested that transcript levels in leaves and flowers were not significantly different (Welchen et al., 2002). This apparent discrepancy with respect to GUS activity levels described here can be explained because northern analysis was performed on total RNA extracted from rosette leaves and flowers at different developmental stages (i.e. the entire rosette or inflorescence), while only mature leaves and open flowers were used here for the GUS activity measurements. Indeed, GUS activity driven by the *Cytc-1* promoter decreases with leaf development and increases with flower development, as indicated by histochemical assays.

Regarding *Cytc-2* gene expression, we have previously failed to observe a specific distribution of transcripts in flowers by in situ hybridization (Ribichich et al., 2001), although the presence of the mRNA could readily be observed in northern analyses (Welchen et al., 2002). Considering the results obtained in this study, which shows that the *Cytc-2* promoter directs preferential GUS expression in anthers, the negative results of in situ hybridization experiments most likely reflect experimental problems with the probe used. In agreement, longer exposure times are required to detect *Cytc-2* transcript levels with this probe in northern blots in comparison to those required for *Cytc-1*.

We conclude, then, that GUS activity measurements are good indicators of high *Cytc* promoter activity and gene expression, and that regulation of the expression of both *Cytc* genes in specific cell types occurs, at least

in part, at the transcriptional level. This is also true for the induction by Suc and cytokinin. We cannot rule out, however, the existence of post-transcriptional processes that adjust steady-state transcript levels in defined organs. As an example, there is not an absolute correlation of transcript (Welchen et al., 2002) and GUS levels in roots and siliques for both genes. Besides post-transcriptional effects, this could be due to the fact that relevant promoter elements, located either upstream or downstream, are lacking from our constructs. In the case of the *Cytc-1* gene, it seems unlikely that upstream sequences are missing, since the entire intergenic region was analyzed.

By performing a set of deletions and site-directed mutations, we have obtained evidence that a region containing two elements with the sequence TGGGCC/T, named site II elements (Kosugi et al., 1995; Kosugi and Ohashi, 1997; Trémousaygue et al., 2003), is required for expression of the *Cytc-1* gene. These elements are usually found in a similar location in promoters of genes that are actively expressed in cycling cells, such as proliferating cell nuclear antigen and ribosomal protein genes, and have been described as binding sites for transcription factors of the TCP-domain family (Cubas et al., 1999). According to the mutagenesis experiments, among the two site II elements present in the *Cytc-1* promoter, only the one located more proximal to the start codon seems to be essential for expression. This element contains the sequence GGGCCCAT (ATGGGCCC in the complementary strand), which more closely matches the consensus sequences GGNCCCAC and GTGGNCCC deduced by site-selection studies as preferred binding sites of different members of the TCP-domain family (Kosugi and Ohashi, 2002).

Site II elements are frequently associated with downstream internal telomeric repeats or *telo*-boxes, which seem to act as stimulatory elements (Manevski et al., 2000). This arrangement is also observed in the *Cytc-1* promoter. Indeed, mutation of the *Cytc-1 telo*-box originates a severe decrease in expression, producing plants with low GUS activity only in anthers. These results strongly suggest that expression of the *Cytc-1* gene may be linked to cell division activity through the presence of these elements.

It is noteworthy that the *Cytc-2* gene also contains a pair of closely located site II motifs. However, deletion of a region containing these motifs does not produce a noticeable effect on GUS activity (data not shown). Considering the broader expression pattern observed for the *Cytc-2* gene, this may indicate that other elements are responsible for the observed expression patterns and that the site II motifs are not functional. A different explanation would be that these site II motifs confer expression characteristics that have not been explored here or that their importance is masked by the presence of additional elements that respond to different transcriptional networks. Nevertheless, the conservation of site II motifs in both *Cytc* genes suggests that they may have a role in the coordination of the expression of these genes through common interacting transcription factors. In this sense, the presence of two or more site II motifs between −100 and −400 of the translation start site in promoters of genes encoding components of the cytochrome *c*-dependent respiratory pathway is far from random expectations. An attractive hypothesis would be that a diversity of proteins interacting with these elements may participate as central actors of coordinated regulatory mechanisms involved in the biogenesis of this part of the plant mitochondrial respiratory chain.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Arabidopsis (*Arabidopsis thaliana* L. Heyhn.) ecotype Columbia (Col-0) was purchased from Lehle Seeds. Histochemical and fluorometric GUS assays were performed on plants grown on soil in a growth chamber at 22°C to 24°C under long-day photoperiods (16 h of illumination by a mixture of cool-white and GroLux fluorescent lamps) at an intensity of approximately 200 μE m^{-2} s^{-1}. Seedlings were grown in petri dishes containing Murashige and Skoog medium and 0.8% agar under similar conditions. The effect of Suc and cytokinin on expression was analyzed in dark-grown seedlings. For this purpose, either 3% Suc or 50 μM BAP, as indicated, were added to the culture medium. The dishes were kept at 4°C for 4 d and then transferred to growth chamber conditions and kept under complete darkness for 14 d.

Reporter Gene Construct and Plant Transformation

A 1.23-kb *Nhe*I/*Bgl*II fragment from the *Cytc-1* gene (At1g22840), comprising nucleotides −1,182 to +54 with respect to the ATG initiation codon, was cloned in frame in the *Xba*I and *Bam*HI sites of plasmid pBI101.3. Constructs containing upstream deletions were obtained by PCR amplification with oligonucleotide CATP16 and either CATP15, CATP13, CATP19, CATP17, or CATP11 (Table II) followed by cloning in the *Hin*dIII and *Bam*HI sites of pBI101.3. A deletion comprising nucleotides −147 to −218 was constructed using partially complementary primers that hybridized at both sides of the deleted region. Primers delA1 and delA2 were used with CATP13 and CATP16, respectively, to amplify hybridizing upstream and downstream sequences. The resulting products were mixed in buffer containing 50 mM Tris-HCl (pH 7.2), 10 mM $MgSO_4$, and 0.1 mM dithiothreitol (DTT), incubated at 95°C during 5 min, and annealed by allowing the solution to cool to 24°C in approximately 1 h. After this, 0.5 mM of each dNTP and 5 units of the Klenow fragment of *Escherichia coli* DNA polymerase I were added, and incubation was followed for 1 h at 37°C. A portion of this reaction was directly used to amplify the chimeric fragments using primers CATP13 and CATP16. In a similar way, complementary primers were used to generate scanning mutations along the −147 to −218 promoter region and to mutate specific putative regulatory elements. The sequences of the oligonucleotides used are shown in Table II. All constructs were checked by DNA sequencing.

A fragment spanning nucleotides −967 to +54 of the *Cytc-2* gene (At4g10040) promoter was obtained by PCR amplification of Arabidopsis genomic DNA using primers CATP22 and CATP26. The resulting fragment, containing *Hin*dIII and *Bgl*II sites, was cloned in frame in the *Hin*dIII and *Bam*HI sites of pBI101.3.

The respective constructs were introduced into *Agrobacterium tumefaciens* strain GV2260 and transformed bacteria were used to obtain transgenic Arabidopsis plants by the floral-dip procedure (Clough and Bent, 1998). Transformed plants were selected on the basis of kanamycin resistance and positive PCR carried out on genomic DNA with gene-specific primers and the *gus* primer 5′-TTGGGGTTTCTACAGGAC-3′. Primary transformants for each construct were initially analyzed for GUS expression by histochemistry. From these, 10 independent lines with single insertions (as deduced by kanamycin resistance segregation) and with representative expression patterns (those common to a vast majority of transformants) were further reproduced and homozygous T3

and T4 plants were used to analyze *gus* expression. Plants transformed with pBI101.3 or pBI121 were obtained in a similar way.

GUS Assays

GUS activity of transgenic plants was analyzed by histochemical staining using the chromogenic substrate X-gluc as described by Hull and Devic (1995). Whole plants or separated organs were immersed in a 1 mM X-gluc solution in 100 mM sodium phosphate, pH 7.0, and 0.1% Triton X-100, and, after applying vacuum for 5 min, they were incubated at 37°C until satisfactory staining was observed. Tissues were cleared by immersing them in 70% ethanol.

Specific GUS activity in protein extracts was measured using the fluorogenic substrate 4-methylumbelliferyl β-D-glucuronide (MUG) essentially as described by Jefferson et al. (1987). Total protein extracts were prepared by grinding the tissues in extraction buffer (50 mM sodium phosphate, pH 7.0, 10 mM EDTA, 10 mM β-mercaptoethanol) containing 0.1% (w/v) SDS and 1% Triton X-100, followed by centrifugation at 13,000*g* for 10 min. GUS activity in supernatants was measured in extraction buffer containing 1 mM MUG and 20% methanol. Reactions were stopped with 0.2 M Na_2CO_3 and the amount of 4-methylumbelliferone was calculated by relating relative fluorescence units with those of a standard of known concentration. The protein concentration of extracts was determined as described by Sedmak and Grossberg (1977).

DNA-Binding Assays

Nuclear extracts were prepared from cauliflower buds (obtained from a local market) as described by Manzara and Gruissem (1995). Clones expressing recombinant proteins AtPuralpha and AtTCP20 fused to the *E. coli* maltose-binding protein were kind gifts of Dr. Bernard Lescure and Dr. Dominique Trémousaygue (CNRS-INRA, Castanet-Tolosan, France). Expression and purification of the recombinant proteins were carried out as indicated by the manufacturers of the pMAL-c2 system (New England Biolabs). For electrophoretic mobility shift assays, aliquots of extracts or purified proteins were incubated with double-stranded DNA (25,000 counts per minute) obtained by amplification of the corresponding fragments with primer pairs CATP16/CATP17, CATP16/CATP11, or CATP12/CATP17, followed by restriction enzyme cleavage and labeling with [α-^{32}P]dATP by filling in the 3′-ends using the Klenow fragment of DNA polymerase. For nuclear extracts, binding reactions (20 μL) contained, in addition to labeled DNA, 20 mM HEPES (pH 7.5), 50 mM KCl, 2 mM $MgCl_2$, 0.5 mM EDTA, 1.0 mM DTT, 0.5% Triton X-100, 10% glycerol, 1.5 μg poly(dI-dC), and 2 μg pBI221 (as nonspecific competitors). Reactions were incubated for 20 min on ice, supplemented with 2.5% Ficoll, and immediately loaded onto a running gel (5% acrylamide, 0.08% bis-acrylamide in 0.5× TBE plus 2.5% glycerol; 1× TBE is 90 mM Tris-borate, pH 8.3, 2 mM EDTA). The gel was run in 0.5× TBE at 30 mA and 4°C for 1.5 h and dried prior to autoradiography. For recombinant proteins, binding reactions contained 10 mM Tris-HCl (pH 7.5), 1.0 mM DTT, 10% glycerol, 22 ng/μL bovine serum albumin, and 1 μg poly(dI-dC), and incubation and electrophoresis were carried out at room temperature.

Distribution of Materials

Upon request, all novel materials described in this publication will be made available in a timely manner for noncommercial research purposes, subject to the requisite permission from any third-party owners of all or part of the material. Obtaining any permissions will be the responsibility of the requester.

ACKNOWLEDGMENTS

We thank Dr. Bernard Lescure and Dr. Dominique Trémousaygue (CNRS-INRA, Castanet-Tolosan, France) for sending us clones expressing proteins AtPuralpha and AtTCP20. We also thank Raquel Chan and two anonymous reviewers for helpful comments and suggestions, and Ivana Viola for cloning the *Cytc-2* promoter fragment.

Received May 20, 2005; revised June 30, 2005; accepted July 6, 2005; published August 19, 2005.

LITERATURE CITED

Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J **16:** 735–743

Cubas P, Lauter N, Doebley J, Coen E (1999) The TCP domain: a motif found in proteins regulating plant growth and development. Plant J **18:** 215–222

Curi GC, Welchen E, Chan RL, Gonzalez DH (2003) Nuclear and mitochondrial genes encoding cytochrome *c* oxidase subunits respond differently to the same metabolic factors. Plant Physiol Biochem **41:** 689–693

Elorza A, León G, Gómez I, Mouras A, Holuigue L, Araya A, Jordana X (2004) Nuclear *SDH2-1* and *SDH2-2* genes, encoding the iron-sulfur subunit of mitochondrial complex II in Arabidopsis, have distinct cell-specific expression patterns and promoter activities. Plant Physiol **136:** 4072–4087

Felitti SA, Chan RL, Gago G, Valle EM, Gonzalez DH (1997) Expression of sunflower cytochrome *c* mRNA is tissue-specific and controlled by nitrate and light. Physiol Plant **99:** 342–347

Felitti SA, Gonzalez DH (1998) Carbohydrates modulate the expression of the sunflower cytochrome *c* gene at the mRNA level. Planta **206:** 410–415

Hamilton DA, Schwarz YH, Mascarenhas JP (1998) A monocot pollen-specific promoter contains separable pollen-specific and quantitative elements. Plant Mol Biol **38:** 663–669

Hanson MR (1991) Plant mitochondrial mutations and male sterility. Annu Rev Genet **25:** 461–486

Heiser V, Brennicke A, Grohmann L (1997) The plant mitochondrial 22 kDa (PSST) subunit of respiratory chain complex I is encoded by a nuclear gene with enhanced transcript levels in flowers. Plant Mol Biol **31:** 1195–1204

Huang J, Struck F, Matzinger DF, Levings CS III (1994) Flower-enhanced expression of a nuclear-encoded mitochondrial respiratory protein is associated with changes in mitochondrion number. Plant Cell **6:** 439–448

Hull GA, Devic M (1995) The beta-glucuronidase (*gus*) reporter gene system. Gene fusions, spectrophotometric, fluorometric, and histochemical detection. *In* H Jones, ed, Methods in Plant Molecular Biology, Vol 49: Plant Gene Transfer and Expression Protocols. Humana Press, Totowa, NJ, pp 125–141

Jang I-C, Choi W-B, Lee K-H, Song SI, Nahm BH, Kim J-K (2002) High-level and ubiquitous expression of the rice cytochrome *c* gene *OsCc1* and its promoter activity in transgenic plants provides a useful promoter for transgenesis of monocots. Plant Physiol **129:** 1473–1481

Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J **20:** 3901–3907

Kosugi S, Ohashi Y (1997) PCF1 and PCF2 specifically bind to *cis* elements in the rice proliferating cell nuclear antigen gene. Plant Cell **9:** 1607–1619

Kosugi S, Ohashi Y (2002) DNA binding and dimerization specificity and potential targets for the TCP protein family. Plant J **30:** 337–348

Kosugi S, Suzuka I, Ohashi Y (1995) Two of three promoter elements identified in a rice gene for proliferating cell nuclear antigen are essential for meristematic tissue-specific expression. Plant J **7:** 877–886

Lee SLJ, Warmke HE (1979) Organelle size and number in fertile and T-cytoplasmic male-sterile corn. Am J Bot **60:** 141–148

Mackenzie S, McIntosh L (1999) Higher plant mitochondria. Plant Cell **11:** 571–586

Manevski A, Bertoni G, Bardet C, Tremousaygue D, Lescure B (2000) In synergy with various *cis*-acting elements, plant interstitial telomere motifs regulate gene expression in *Arabidopsis* root meristems. FEBS Lett **483:** 43–46

Manzara T, Gruissem W (1995) Identification of promoter sequences that interact with DNA-binding proteins. *In* P Maliga, DF Klessig, AR Cashmore, W Gruissem, JE Varner, eds, Methods in Plant Molecular Biology. A Laboratory Course Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 233–260

Mascarenhas JP, Hamilton DA (1992) Artifacts in the localization of GUS activity in anthers of petunia transformed with a CaMV 35S-GUS construct. Plant J **2:** 405–408

Ribichich K, Tioni MF, Chan RL, Gonzalez DH (2001) Cell-type specific expression of plant cytochrome *c* mRNA in developing flowers and roots. Plant Physiol **125:** 1603–1610

Sedmak J, Grossberg S (1977) A rapid, sensitive, and versatile assay for protein using Coomassie Brilliant Blue G-250. Anal Biochem **79:** 544–552

Smart C, Monéger F, Leaver CJ (1994) Cell-specific regulation of gene expression in mitochondria during anther development in sunflower. Plant Cell **6:** 811–825

Thirkettle-Watts D, McCabe TC, Clifton R, Moore C, Finnegan PM, Day DA, Whelan J (2003) Analysis of the alternative oxidase promoters from soybean. Plant Physiol **133:** 1158–1169

Trémousaygue D, Garnier L, Bardet C, Dabos P, Hervé C, Lescure B (2003) Internal telomeric repeats and ''TCP-domain'' protein binding sites co-operate to regulate gene expression in *Arabidopsis thaliana* cycling cells. Plant J **33:** 957–966

Trémousaygue D, Manevski A, Bardet C, Lescure N, Lescure B (1999) Plant interstitial telomere motifs participate in the control of gene expression in root meristems. Plant J **20:** 553–561

Welchen E, Chan RL, Gonzalez DH (2002) Metabolic regulation of genes encoding cytochrome *c* and cytochrome *c* oxidase subunit Vb in Arabidopsis. Plant Cell Environ **25:** 1605–1615

Welchen E, Chan RL, Gonzalez DH (2004) The promoter of the *Arabidopsis* nuclear gene *COX5b-1*, encoding subunit 5b of the mitochondrial cytochrome *c* oxidase, directs tissue-specific expression by a combination of positive and negative regulatory elements. J Exp Bot **55:** 1997–2004

Zabaleta E, Heiser V, Grohmann L, Brennicke A (1998) Promoters of nuclear-encoded respiratory chain complex I genes from *Arabidopsis thaliana* contain a region essential for anther/pollen-specific expression. Plant J **15:** 49–59