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Identification of Enhancer Elements in the Upstream Region of the Nuclear Photosynthetic Gene *ST-LS1*

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The nuclear gene *ST-LS1* from potato encodes a 10-kilodalton protein that is a component of the oxygen-evolving complex of photosystem II. Analysis of the expression of a reporter gene driven by chimeric promoters, consisting of *ST-LS1* upstream sequences and a truncated cauliflower mosaic virus 35S promoter, suggests that a strong positive regulatory element is located between position −345 and −261, whereas both the region −261 to +11 and the more upstream region −1600 to −530 are devoid of autonomous strong positive elements detectable by this approach. The *ST-LS1* upstream region contains redundant elements conferring light-regulated and organ-specific expression, one of them being located between position −130 and +11. In addition, enhancer-like sequences conferring light-regulated as well as organ-specific expression to heterologous promoters were identified. These sequences are functional not only when located 5′-upstream of the coding region but also when placed 3′-downstream of the polyadenylation signal, thus representing one of the first examples of a plant gene-derived enhancer being able to induce a truncated heterologous promoter from a position 3′-downstream of the transcription unit.

INTRODUCTION

The analysis of light-regulated genes encoding proteins involved in photosynthesis has been performed using mainly *rbcS* and *Cab* gene families. Although the main expression characteristics of these gene families are very similar, they are differentially regulated by light quality as well as light intensities and differ in their diurnal expression patterns (Kaufmann, Thompson, and Briggs, 1984; Tobin and Silverthorne, 1985; Piechulla and Gruissem, 1987). These observations, as well as the lack of obvious homologies in their upstream sequences, indicate that different mechanisms might be involved in the regulation of photosynthetic genes. To extend the analysis to other light-inducible genes, we analyzed *cis*-active elements essential for the specific expression of the nuclear gene *ST-LS1* of potato (Eckes, Schell, and Willmitzer, 1985; Eckes et al., 1986). In contrast to the *rbcS* and *Cab* genes, the *ST-LS1* gene is present as a single copy gene in the potato genome (Eckes et al., 1986), resulting in a considerable advantage for the analysis of the *ST-LS1* regulatory elements. The *ST-LS1* gene product is transported into the thylakoid lumen and is associated with the oxygen-evolving complex of photosystem II (Lautner et al., 1988). Previous analysis revealed that a 345-bp-long DNA fragment upstream of the transcription initiation site is sufficient for high-level and specific expression of this chimeric gene (Stockhaus et al., 1987). Our first goal was a more precise localization of essential *cis*-acting elements of the *ST-LS1* upstream region. To this end, different subfragments of the *ST-LS1* upstream region were fused to a truncated 35S promoter and the chloramphenicol acetyltransferase (CAT) coding sequence as a reporter gene. In this connection we wanted to elucidate whether the proximal *ST-LS1* upstream sequences from the transcription initiation site to position −345 contain redundant regulatory elements. Furthermore, we wanted to see whether far-upstream elements play an important role in the *ST-LS1* regulation.

It has been shown that the *ST-LS1* promoter region from position −130 to +11 is not sufficient to drive a detectable expression of a fused *CAT* gene (Stockhaus et al., 1987). By adding a 35S enhancer element to the 5′-end of the −130 deletion, we tested whether this promoter fragment contains elements that might be involved in the tissue-specific and light-dependent expression of the *ST-LS1* gene.

In view of the fact that, for most plant genes analyzed, the corresponding enhancer elements have only been demonstrated to be active when inserted 5′-upstream of the transcription unit, several constructs were analyzed in transgenic tobacco plants with the elements to be tested for enhancer activity located 3′-downstream of the transcription unit. The data described in Results clearly demonstrate that the 5′-upstream region of the *ST-LS1* gene

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contains an enhancer element which activates both homologous and *heterologous* truncated promoters even when placed 3′-downstream of the transcription unit, which represents a unique observation with respect to plant nuclear genes.

RESULTS

Positive Regulatory Elements Are Present in the 5′ Vicinity of the Transcription Initiation Site, but not in the Far-Upstream Region

As a first step in the analysis, we wanted to identify positive regulatory elements of the *ST-LS1* upstream region. To this end, different 5′-upstream fragments were fused to a 35S promoter truncated at position −90 and a reporter gene, consisting of a CAT coding sequence and a polyadenylation signal derived from the transfer-DNA (T-DNA) gene 7 (Velten and Schell, 1985). These chimeric genes were integrated into the tobacco genome via *Agrobacterium* transformation. The expression analysis of genes introduced into the plant genome is complicated by the fact that the expression level of introduced genes varies in independent transformants (Jones, Dunsmuir, and Bedbrook, 1985; Nagy et al., 1985; Eckes et al., 1986). Therefore, leaves of 10 to 30 tobacco plants, transformed with each of the constructs and shown to express the cotransformed nopaline synthase gene, were analyzed for CAT enzyme activity individually (Gorman, Mofat, and Howard, 1982). The average conversion rates were normalized relative to the average conversion rate conferred by the construct containing the longest upstream element (Figure 1, Δ673). Figure 1 presents the different chimeric genes and the relative conversion rates determined by CAT enzyme assays. The experiments revealed that the upstream elements from −673 to −96 (Figure 1, construct Δ673) as well as from −345 to −96 (Figure 1, Δ345) are sufficient for a high-level expression of the chimeric genes in leaves. The Δ345 construct is expressed to an average level of about 80% of the Δ673 expression. A further deletion at the 5′-end to position −261 (Figure 1, Δ261) decreased the average conversion rate to 1.5%, which is almost the background level of CAT activity. This result indicates that the region from −345 to −261 contains at least one strong, positive, regulatory element, essential for the high-level expression of the *ST-LS1* gene. Further deletions from the 5′-end decreased the expression of the corresponding chimeric genes almost to the level of detection (Figure 1, constructs Δ170 and Δ140) except for deletion Δ210 (Figure 1, construct Δ210). In leaf tissue of transgenic plants containing the Δ210 construct, we detected an average conversion rate of 13%, which is a

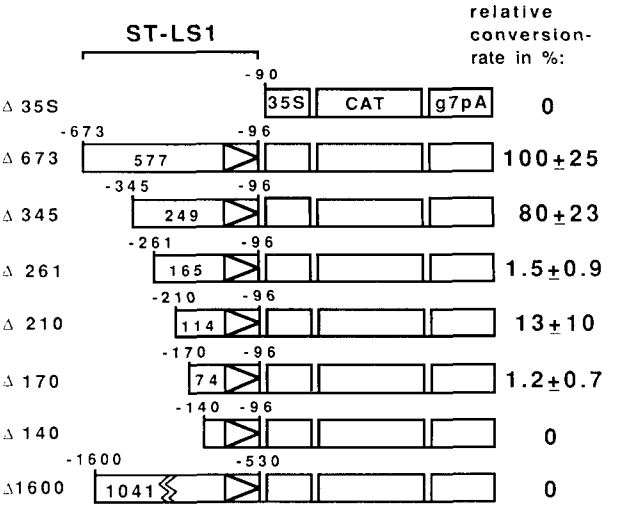

Figure 1. A Strong, Positive Regulatory Element Is Located between Position −261 and −345 of the *ST-LS1* Upstream Region.

Several DNA fragments derived from the *ST-LS1* upstream region were fused to a 35S promoter truncated at position −90 and a CAT coding region (CAT) followed by a polyadenylation signal derived from the T-DNA gene 7 (g7pA). For cloning of these upstream fragments, appropriate restriction sites were used. For each construct, leaves of 10 to 30 plants were assayed for CAT enzyme activity individually. The average conversion rate is given in percentage of the conversion rate determined for construct Δ673. The ± values indicate the standard errors. The size (in base pairs) of the fragments used is indicated in the boxes.

significantly higher level when compared to the other deletions, although the standard deviation is fairly large.

We also wanted to determine whether the *ST-LS1* gene also contains far-upstream sequences that have been found to be essential for maximal expression levels of *rbcS* and *Cab* genes (Timko et al., 1985; Castresana et al., 1988; Poulsen and Chua, 1988). To this end, a fragment from −1600 to −530 of the *ST-LS1* gene was added to the truncated 35S promoter and the *CAT* gene (Figure 1, construct Δ1600). Only background levels of CAT activity were detectable in all 26 transgenic tobacco plants containing this chimeric gene.

Identification of *ST-LS1* Enhancer-Like Elements Conferring Tissue-Specific Expression

It has been shown that certain upstream elements of light-regulated nuclear genes have enhancer-like properties. These elements enhance transcription when fused to truncated promoters in either orientation (Timko et al., 1985; Simpson, et al., 1986; Nagy et al., 1987). The data shown

in Figure 1 reveal that three constructs (Δ673, Δ345, and Δ210) enhance the expression from a fused truncated 35S promoter. To determine whether or not these enhancer elements also confer tissue specificity to the expression, they were fused in either orientation to this truncated 35S promoter (Figure 2A). The results of the CAT assays performed for each construct with leaf and root extracts of several transgenic plants are summarized in Figure 2A. For the truncated 35S promoter construct we did not detect any CAT activity in leaf and root extracts of six transgenic tobacco plants (Figure 2A, Δ35S). In all seven transgenic Δ673 plants containing the −673 to −96 fragment in the same orientation as in the *ST-LS1* upstream region (correct orientation) (Figure 2A, Δ673), we detected a high level of CAT expression in leaf extracts (Figure 2B, panels 1 and 2). Figure 2B shows representative results of the determination of the CAT enzyme activities. In two of the plants, there was a low level of CAT activity detectable in roots (Figure 2B, panel 1), whereas, in root tissue of the other five plants, we did not detect any CAT activity (Figure 2B, panel 2). Five transgenic plants containing this fragment (−673 to −96) in the reverse orientation (Figure 2A, Δ673rev) expressed the *CAT* gene in a leaf-specific manner (Figure 2B, panel 3), which agrees with our earlier observation (Stockhaus et al., 1987). The constructs Δ345 and Δ210 contain 5′-deleted upstream elements in the correct orientation (Figure 2A, Δ345 and Δ210). We analyzed the CAT expression in leaf and root tissue of six Δ345 and eight Δ210 transformed tobacco plants.

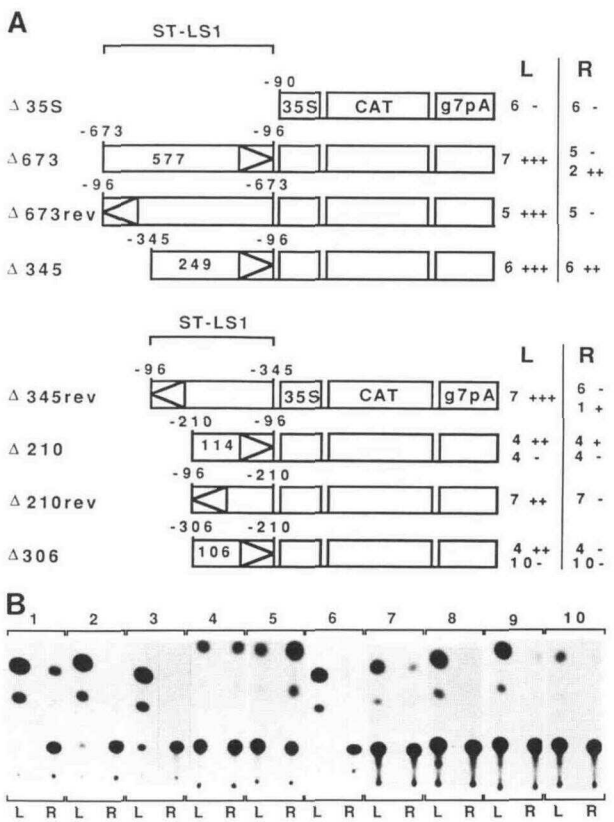

Figure 2. Upstream Sequences of the *ST-LS1* Gene Contain Small Enhancer-Like Elements.

(A) Three fragments derived from the *ST-LS1* upstream region were fused in either orientation to a 35S promoter truncated at position −90 and a *CAT* reporter gene (CAT) followed by the polyadenylation signal of the T-DNA gene 7 (g7pA). The activity of the CAT enzyme was determined in leaf (L) and root (R) tissue of several plants for each construct. The number is given on the right side next to the construct together with CAT activity (+++ corresponds to the highest expression observed).

(B) Representative CAT assays for different constructs. Panels 1 and 2, construct Δ673; panel 3, construct Δ673rev; panels 4 and 5, construct Δ345; panel 6, construct Δ345rev; panel 7, construct Δ210; panel 8, construct Δ210rev; panels 9 and 10, construct Δ306.

In all plants analyzed, both constructions were not only expressed in leaves, but also in root tissue (Figure 2A, Δ345 and Δ210; Figure 2B, panels 4, 5, and 7). When seven transgenic plants for each of the same elements in the reverse orientation (Figure 2A, Δ345rev and Δ210rev) were analyzed, a leaf-specific expression was observed for 13 plants (Figure 2B, panels 6 and 7). One of seven plants containing the construct Δ345rev expressed the CAT enzyme in roots (data not shown). All three different fragments used (Δ673, Δ345, and Δ210) thus have the properties of enhancers since, upon fusion 5′ to the truncated 35S promoter in either orientation, they activate this otherwise inactive promoter. The fragment from position −210 to −96 results in the weakest, but nevertheless specific, expression. In this respect, it should be noted that only about 50% of the Δ210 plants analyzed for CAT activity expressed the CAT enzyme at a detectable level, whereas, in the case of the Δ673 and Δ345 constructs, a high level was observed in almost all plants tested. It is noteworthy that particularly the two shorter elements used (Δ345 and Δ210) did not confer leaf-specific expression when fused in the right orientation to the truncated 35S promoter but only when present in the reverse orientation. The possible reasons for this will be discussed later.

To see whether the *ST-LS1* proximal upstream sequence is composed of independent redundant elements, a fragment from −306 to −210 was fused to the truncated 35S promoter (Figure 2A, Δ306). Out of 14 plants analyzed, only four plants expressed a low amount of CAT enzyme in leaves. These four plants expressed the *CAT* gene in a leaf-specific manner with very low expression levels in root extracts (Figure 2B, panels 9 and 10). We cannot exclude that the low level of expression of the Δ306 construct is due to a position effect (e.g., integration near a strong enhancer).

A Region of the *ST-LS1* Promoter Containing Homology to the Box II Element Is not Sufficient for Expression

We reported earlier that the *ST-LS1* upstream region from −130 to +11 is not sufficient for the expression of a fused *CAT* gene (Stockhaus et al., 1987). This result does not exclude the possibility that the promoter fragment concerned contains negative regulatory elements that, in the natural sequence context, cooperate with an enhancer, thereby resulting in a specific pattern of expression. One candidate for a positive regulatory element in the *ST-LS1* upstream region would be a sequence from position −157 to −143 containing a motif in which 11 out of 14 nucleotides (Stockhaus et al., 1987) are homologous to the box II element of an *rbcS* gene (Fluhr et al., 1986). It has been demonstrated that this box II can act as a negative regulatory element. On the other hand, it also seems to be involved in the positive regulation of the pea *rbcS-3A* gene (Kuhlemeier et al., 1987; Green et al., 1988). To see whether the region from position −174 to −130, including the sequence of the *ST-LS1* gene homologous to box II, is sufficient to obtain an active *ST-LS1* promoter, the upstream fragment from position −174 to +11 was fused to the *CAT* reporter gene (Figure 3A, construct E). In leaf extracts from 19 transgenic tobacco plants, we did not detect any CAT enzyme activity, indicating that the box II homology is not sufficient. This result demonstrates that the strong, positive regulatory elements of the *ST-LS1* gene are located upstream of position −174, which is in agreement with the data presented in Figures 1 and 2, locating a strong, positive regulatory element in the region from −345 to −261.

The *ST-LS1* Promoter Region from −130 to +11 Contains Elements Sufficient for Leaf-Specific and Light-Induced Expression

To determine whether or not the −130 to +11 promoter sequence contains elements important for the specificity of the *ST-LS1* gene expression, we decided to add the enhancer of a "constitutive" promoter to the *ST-LS1* promoter. Figure 3 presents the different constructs, the analysis of the tissue-specific expression on the level of CAT enzyme activity, and the analysis of the light-dependent expression on the steady-state RNA level. A 35S enhancer fragment (−382 to −32) was added in front of a *CAT* reporter gene driven by the *ST-LS1* promoter truncated at position −130 (Figure 3A). To vary the strength of the 35S enhancer, it was inserted in either orientation and as a tandem repeat (Figure 3A, constructs A, B, and C). The expression analysis of six, five, and four tobacco plants transformed with constructs A, B, and C, respectively, revealed that all constructs result in a high level of expression in leaves, without CAT activity being detectable

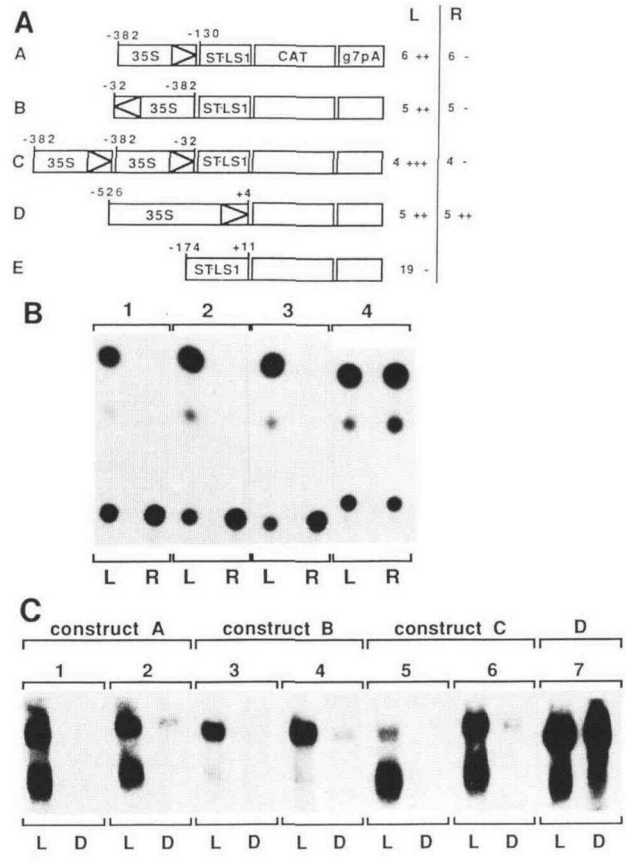

Figure 3. The *ST-LS1* Promoter Fragment from Position −130 to +11 Contains Elements That Are Sufficient for Specific Regulation.

(A) A 35S enhancer sequence from position −382 to −32 was fused in either orientation and as a tandem repeat to the *ST-LS1* promoter truncated at position −130 (constructs A, B, and C). The 35S promoter from position −526 to +4 fused to the same reporter gene serves as a control (construct D). Construct E contains the *ST-LS1* promoter from position −174 to +11 fused to the *CAT* reporter gene. The expression levels in leaf (L) and root (R) tissue are indicated by + and −.

(B) Representative CAT assays performed with leaf (L) and root (R) tissue of transgenic plants containing constructs A to *D* are shown. Panel 1, construct A; panel 2, construct B; panel 3, construct C; panel 4, construct D.

(C) RNA gel blot analysis of leaf RNA (50 μg per lane) isolated from transgenic tobacco plants containing constructs A to D. The plants were cultivated on 2MS medium and kept either in the dark for 7 days (D) or under the normal 16-hr light/8-hr dark (L) regime. A mixed probe consisting of the CAT coding region and the *ST-LS1* cDNA was used for the hybridization. The upper band represents the *CAT* mRNA, whereas the lower band corresponds to the transcripts of the endogenous *ST-LS1* gene serving as an internal control for the light/dark regulation of the expression. The weak *ST-LS1* signal in lanes 3 and 4 is due to a weak *ST-LS1* probe. Representative results are shown. Panels 1 and 2, construct A; panels 3 and 4, construct B; panels 5 and 6, construct C; panel 7, construct D.

in root tissue of all plants analyzed (Figure 3B, panels 1 to 3). A *CAT gene fused to a 35S promoter served as a* control (Figure 3A, construct D). This chimeric gene is expressed to a high level in both leaves and roots (Figure 3B, panel 4).

As outlined in the Introduction, the expression of the *ST-LS1* gene is light-dependent. To test whether or not the −130 to +11 fragment also contains elements involved in the light response, plants cultivated on 2MS medium (Murashige and Skoog, 1962) were kept in the dark for 7 days, while parallel to this, control plants were kept under the normal light/dark regime. The expression of the different constructs was analyzed by assaying steady-state RNA level by RNA gel blots. The *ST-LS1* cDNA and the CAT coding sequence were used as hybridization probes. The *ST-LS1* probe serves as an internal control and allows the comparison of the expression of the chimeric genes with the expression of the endogenous *ST-LS1* gene of tobacco. Representative results of this analysis are shown in Figure 3C. The upper band corresponds to the transcripts of the chimeric gene and the lower band represents the *ST-LS1* transcripts. The steady-state RNA level of the three different constructs (Figure 3A, constructs A to C) is clearly reduced in the dark-adapted plants as compared to the control plants (Figure 3C, panels 1 to 6), whereas the steady-state RNA level of a *CAT* gene driven by the 35S promoter (Figure 3A, construct D), is not influenced significantly by the dark adaptation of the plants (Figure 3C, panel 7).

These results demonstrate that the *ST-LS1* −130 to +11 promoter fragment contains elements conferring tissue-specific and light-dependent expression when they are combined with a "constitutive" 35S enhancer.

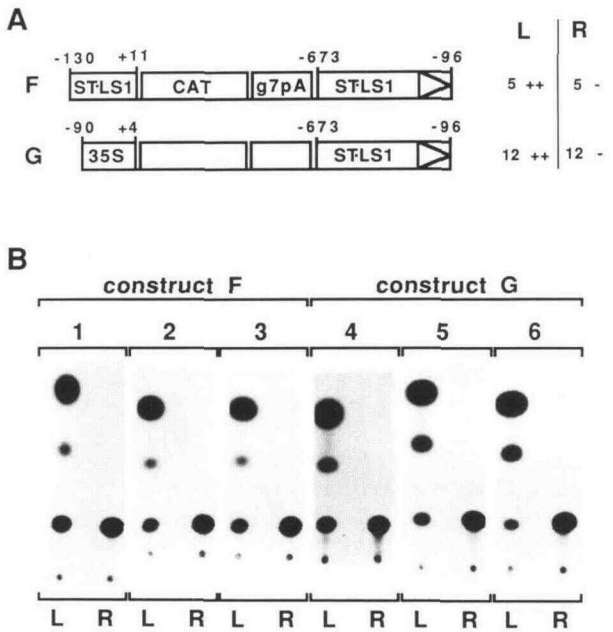

Figure 4. The *ST-LS1* Element from Position −673 to −96 Functions as a Tissue-Specific Enhancer When Placed 3′ of the Polyadenylation Signal.

(A) The *ST-LS1* enhancer element was fused 3′ of the gene 7 polyadenylation signal (g7pA) of a *CAT* gene (CAT) driven by a *ST-LS1* promoter truncated at position −130 (construct F) or a 35S promoter truncated at position −90 (construct G). The results of the CAT expression analysis in leaves (L) and roots (R) of several independent transgenic tobacco plants are shown.
(B) Representative CAT assays are shown. Panels 1 to 3, construct F; panels 4 to 6, construct G.

The *ST-LS1* Enhancer Is Still Functional when Located 3′-Downstream of the Polyadenylation Signal

To test whether the promoter region of the *ST-LS1* gene between position −673 and −96 [thus comprising the region acting as an enhancer when located 5′ of the truncated 35S promoter (cf. Figure 1)] also increases the expression from a position 3′ of the polyadenylation signal of the gene, this enhancer was inserted 3′-downstream of a *CAT* gene driven either by a truncated *ST-LS1* (Figure 4A, construct F) promoter, which previously has been demonstrated to be inactive in transgenic plants (Stockhaus et al., 1987), or a truncated 35S promoter (Figure 4A, construct G). Figure 4 shows the two different chimeric genes and representative results of the analysis of the tissue-specific expression on the level of CAT enzyme assays. Transgenic tobacco plants containing these constructs were analyzed for CAT expression in leaves and roots. In five plants containing construct F (representative results in Figure 4B, panels 1 to 3) and 12 plants containing construct G (representative results in Figure 4B, panels 4 to 6), we detected variable levels of CAT activity in leaves and no CAT activity in root tissue. The *ST-LS1* enhancer thus induces a leaf-specific high level of expression even when positioned at the 3′-end of the gene. We have shown earlier that the *ST-LS1* upstream region from −130 to +11 contains regulatory elements. These elements are dispensible for the effect of the *ST-LS1* enhancer, as they can be substituted by a truncated 35S promoter (Figure 4B, panels 4 to 6).

DISCUSSION

The *ST-LS1* gene is a nuclear gene encoding a chloroplast-located protein involved in photosynthesis and thus belongs to the same class of genes as the *rbcS* and *Cab* genes. Within 350 bp upstream of the transcription start

site of both *rbcS* and *Cab* genes, small enhancer-like elements conferring tissue-specific and light-regulated expression were identified (Fluhr et al., 1986; Simpson, Van Montagu, and Herrera-Estrella, 1986; Simpson et al., 1986; Kuhlemeier et al., 1987; Nagy et al., 1987).

A comparable pattern is observed for the *ST-LS1* promoter. Thus, a strong, positive element must be located in the region between position −345 and −261, as deletion of this region almost completely abolishes expression. A construct containing a fragment from −306 to −210 is expressed at low levels, indicating that the upstream region from position −306 to −261 does not contain a complete, positive regulatory element. The expression level conferred by the −673 to −96 sequence is significantly higher than the expression of the −345 to −96-driven construct, indicating that there is probably another positive element located in the region from −673 to −345. Thus, these data suggest that the *ST-LS1* promoter contains redundant, positive regulatory elements located in the region from −530 to −261. Furthermore, these experiments give some indication for the presence of a negative element located between position −261 and −210, as a deletion of this fragment resulted in a significant increase of the *CAT* gene expression. Further deletions to positions −170 and −140 suppressed the *CAT* gene expression almost beneath the level of detection, indicating that there are no positive elements located in the region from position −174 to −96 that autonomously are sufficient for promoter activity.

Kuhlemeier et al. (1988) described that a 170-bp-long DNA fragment upstream of the transcription initiation site of the pea *rbcS-3A* gene is sufficient for specific expression of this gene. This *rbcS* promoter fragment contains a sequence motif designated box II (Fluhr et al., 1986), which has been shown to be essential for the transcription from the *rbcS-3A* promoter truncated at position −170 (Green et al., 1988; Kuhlemeier et al., 1988).

Although the *ST-LS1* promoter fragment from −174 to +11 includes a motif highly homologous to the box II element [i.e., 11 out of 14 nucleotides are homologous (compare Stockhaus et al., 1987)], this is not sufficient to obtain detectable expression of the fused *CAT* reporter gene. One possible explanation is that the function of the box II sequence is not of equal importance to all light-regulated genes. It has been reported that, for the expression of *rbcS* and *Cab* genes, so-called far-upstream sequences are necessary (Simpson et al., 1985; Timko et al., 1985; Castresana et al., 1988; Poulsen and Chua, 1988). In case of the *ST-LS1* gene, the strongest positive regulatory elements are clustered in the region from −345 to −261, and no evidence for the presence of independent positive elements located far upstream (position −530 to −1600) was obtained.

One remarkable feature of several constructs containing upstream fragments of the *ST-LS1* promoter fused to the truncated (Δ-90) 35S promoter is the influence of the orientation of the *ST-LS1*-derived fragment on the expression specificity. Thus, exclusive leaf-specific expression was only observed when the *ST-LS1*-derived fragment was fused in the inverse orientation to the truncated promoter, whereas fusions in the correct orientation led to expression in both leaves and roots in a variable number of transformants (cf. Figure 2). One possible explanation for this result is that, in all fusions done in the correct orientation, a situation is created in which regulatory elements located on the truncated 35S promoter can interact with the *ST-LS1* regulatory elements located in the vicinity of the boundary upstream of position −96, thereby resulting in this unexpected pattern of expression. This idea is supported by the observation that the −90 deletion of the 35S promoter contains two protein binding sites (Prat, Willmitzer, and Sanchez-Serrano, 1989). Furthermore, in contrast to our results obtained in *Nicotiana tabacum*, Poulsen and Chua (1988) have reported that a −90 deletion mutant of a 35S promoter fused to a *CAT* reporter gene results in a weak root-specific expression in transgenic *N. plumbaginifolia* plants. In conclusion, these results suggest that the truncated 35S promoter used contains regulatory elements that might interact with elements located further upstream.

Although the *ST-LS1* promoter region from −130 to +11 is not sufficient to drive the expression of a fused reporter gene in transgenic tobacco plants (Stockhaus et al., 1987), addition of a 35S-derived enhancer 5′-upstream of this truncated promoter leads to a leaf-specific and light-regulated expression, indicating that this region contains elements not sufficient for expression but sufficient for regulation of the expression enhanced by a heterologous element (i.e., the 35S enhancer).

In this connection, it is interesting to note that a promoter fragment of the light-regulated *CAB1* gene from position −124 to +30 is not responsive to phytochrome regulation (Nagy et al., 1987). In this experiment, a 35S enhancer fragment was placed 3′-downstream of the polyadenylation signal to enhance expression from the inactive truncated CAB1 promoter. However, it is not unusual that elements responsible for the specific expression pattern of plant genes are located proximal to the transcription initiation site (compare Morelli et al., 1985; Timko et al., 1985). Furthermore, by using constructs consisting of 35S enhancer elements fused 5′ to short, truncated promoters, it has been demonstrated that a maize ADH1 promoter fragment from position −140 to +106 (Ellis et al., 1987) as well as a soybean leghemoglobin promoter fragment extending up to position −139 (Stougaard et al., 1987) contains regulatory sequences sufficient for the specific expression of these genes. These reports together with the data presented here suggest that elements responsible for the specific expression can be located in the vicinity of the CAAT and TATA promoter signals and that these

elements have the ability to cooperate even with heterologous enhancer sequences located further upstream.

Enhancers in the animal system have been defined operationally by their ability to activate otherwise inactive promoters when placed either 5′ or 3′ to the gene in either orientation (Serfling, Jasin, and Schaffner, 1985). Unlike the situation in mammalian systems, however, in most cases where enhancers have been identified in plant genes, these enhancers have been demonstrated to be active only when positioned 5′-upstream of the promoter. There are only three cases where an activation of gene expression has been reported when an enhancer was placed at a 3′-downstream location. In two of the cases (Timko et al., 1985; Chen, Pan, and Beachy, 1988), however, the level of specific expression reached was only about one-tenth when compared to the expression obtained using a construct where the enhancer was located 5′-upstream of the coding region. Furthermore, this low enhancement was completely lost when the enhancer was put 3′-downstream of the polyadenylation signal (Chen, Pan, and Beachy, 1988). The only enhancer that up to now has been described as leading to a strong induction of expression when placed 3′-downstream of the polyadenylation signal is a large DNA fragment encompassing position −30 up to −941 of the 35S cauliflower mosaic virus promoter (Nagy et al., 1987). In view of these results, chimeric genes with enhancer elements located 3′-downstream of the polyadenylation signal were constructed and tested in transgenic tobacco. The results obtained clearly show that the *ST-LS1* enhancer (position −673 to −96) induces leaf-specific expression from a truncated *ST-LS1* promoter (position −130 to +11) when located 3′-downstream of the polyadenylation signal of a reporter gene. More importantly, however, the *ST-LS1* enhancer positioned 3′-downstream of the transcription unit also results in leaf-specific expression when combined with a heterologous truncated promoter, i.e., the 35S promoter fragment comprising position −90 to +4. This is an example of a strong and specific expression in plants resulting from a nuclear plant gene-derived enhancer located 3′-downstream of the transcription unit driven by either a homologous or heterologous, truncated promoter.

The results described in this paper demonstrate that the arrangement of the *ST-LS1* upstream regulatory elements shares some properties in common with the upstream elements of other photosynthetic genes like the *rbcS* and *Cab* genes. Nevertheless, it is important to note that it was not possible until now to find sequence elements that have a general function in the regulation of different light-regulated, nuclear, photosynthetic genes. Therefore, comparative analyses of regulatory elements of genes that encode protein components of the different protein complexes involved in photosynthesis will be helpful for the analysis of the signal transduction chain involved in the controlled expression of these genes.

METHODS

Recombinant DNA Techniques

Standard procedures were used for recombinant DNA work (Maniatis, Fritsch, and Sambrook, 1982).

Transformation of Tobacco Plants with Various Chimeric Genes

Chimeric genes consisting of various 5′-upstream sequences, the CAT coding region, and the termination region of gene 7 of the transfer DNA (T-DNA) of the Ti plasmid ACH5 (Velten and Schell, 1985) were cloned into the intermediate vector pMPK110 (Eckes et al., 1986) and mobilized into the *Agrobacterium* receptor strain C58C1 (pGV3850^{kan}) as described (Van Haute et al., 1983; Jones, Dunsmuir, and Bedbrook, 1985). To transfer the chimeric genes to tobacco cells, leaf discs of *Nicotiana tabacum* cv SNN were infected with the respective *Agrobacterium* strain (Horsch et al., 1985). Regenerated plants were first tested by nopalin assays (Otten and Schilperoort, 1978) and then by DNA gel blotting for the presence of the transferred DNA and the presence of the intact chimeric genes, respectively. The plants were grown on 2MS medium (Murashige and Skoog, 1962) under a 16-hr light/8-hr dark regime. All manipulations were performed according to standards set by the Bundesministerium für Forschung und Technologie.

Analysis of Transgenic Plants

Isolation of DNA and RNA from transgenic plants and their subsequent analysis by blot hybridizations were performed as described (Eckes et al., 1986; Sanchez-Serrano et al., 1987). The plant material was routinely harvested in the middle of the light period, i.e., at around 2 PM. The protein concentrations of plant extracts were determined as described by Bradford (1976), and equal amounts of protein were assayed for CAT activity essentially as described (Gorman, Mofat, and Howard, 1982). The relative conversion rates were determined by scanning of the chromatography plates with a thin-layer scanner. Leaf material from dark-adapted plants was obtained from plants cultivated for 7 days on MS medium (Murashige and Skoog, 1962), supplemented with 2% sucrose in closed boxes kept in the same growth chamber as the light-illuminated ones, which were cultivated on the same medium under a 16-hr light/8-hr dark regime at a temperature of 25°C. For the light irradiation, Philips TL-D 58 W/84 lamps (7000 lux) were used.

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