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The Rubisco small subunit gene as a paradigm for studies on differential gene expression during plant development

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The ribulose 1,5-bisphosphate carboxylase small subunit (rbcS) in higher plants is encoded by a small multigene family. Members of the gene family contain 1–3 introns. The rbcS mRNA is differentially distributed in various plant organs. It is most abundant in leaves, less so in stems and other photosynthetic organs, and almost undetectable in roots. In leaves, the rbcS mRNA level is greatly increased by light through transcriptional control of the genes. Ti-mediated gene transfer experiments have demonstrated that the pea rbcS–E9 gene retains light-regulated expression in transformed petunia calluses and in leaves of transgenic petunia and tobacco plants. A 33-base pair sequence around the TATA box region has been shown to be involved in the light-inducibility of the rbcS–E9 gene in transformed calluses. In transgenic petunia plants, the experiments thus far have shown that 352 base pairs of 5′ upstream sequence is sufficient for light-inducibility, as well as for leaf-specific expression. Further experiments in progress will help to identify and characterize other cis-acting elements involved in the differential expression of the rbcS genes.

Introduction

The small subunit (rbcS) polypeptide of ribulose 1,5-bisphosphate carboxylase–oxygenase is one of the major proteins in chloroplasts and accounts for 12–15% of the total protein in mesophyll cells. This chloroplast polypeptide is synthesized initially on free cytoplasmic polysomes as a larger precursor that is imported subsequently into the organelle by a post-translational process dependent on ATP (cf. Ellis 1981). The exact function of the rbcS polypeptide has not yet been elucidated but it has been shown to be indispensable for the ribulose 1,5-bisphosphate carboxylase enzymatic activity (Andrews & Ballment 1983; Gatenby *et al.* 1985).

In higher plants, the rbcS polypeptide is encoded by a small, multigene family in the nuclear genome (Berry-Lowe *et al.* 1982; Coruzzi *et al.* 1983; Broglie *et al.* 1983; Dean *et al.* 1985). The expression of rbcS genes is controlled by light as well as cell-type specific factors. Because of the high level of expression, the rbcS genes have been used as a paradigm for studies in plant gene regulation during development. This paper summarizes our present knowledge of the structure of rbcS genes and their mode of regulation.

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Gene structure and linkage

Nuclear genes encoding rbcS polypeptides have been isolated from several monocots and dicots. Nucleotide sequence analyses of these genes have revealed a striking degree of homology in their structural organization. Most dicot rbcS genes characterized to date are interrupted by two introns at identical positions with respect to the coding sequence of the mature rbcS polypeptide (Berry-Lowe *et al.* 1982; Cashmore 1983; Coruzzi *et al.* 1984). Intron I interrupts the mature coding sequence between amino acids 2 and 3; intron II occurs between amino acids 47 and 48. Recently, an rbcS gene of *Nicotiana tabacum* (Mazur & Chui 1985) and of *N. plumbaginifolia* (Poulsen *et al.* 1986) have been reported to contain a third intron between amino acids 65 and 66. In contrast to the dicot rbcS genes, all of the monocot rbcS genes sequenced thus far contain only one intron, which varies in position depending on the species. A wheat rbcS gene contains intron I (Broglie *et al.* 1983), whereas all five *Lemna* rbcS genes characterized so far retain intron II (Wimpee 1984).

Irrespective of their numbers, the introns in monocot and dicot rbcS genes sequenced to date are situated at identical positions in the coding sequence of the mature polypeptide. This observation invites speculations with respect to the evolution of rbcS genes in higher plants. It could be argued that the ancestral rbcS gene of higher plants contained at least three introns, and that during evolution, one or more of these introns were lost. If this model were correct, it should also be possible to find rbcS genes that have introns I and III, introns II and III, only intron III, or even no intron at all. The precise loss of introns from the rbcS gene could have been brought about by reverse transcription of transcripts of the appropriate structures followed by recombinational events. Examples of such 'processed genes' have been found in animal systems (Hollis *et al.* 1982).

Sequence analyses of the various monocot and dicot rbcS genes have generated a wealth of data on the derived amino acid sequences of the mature rbcS polypeptides as well as their transit peptides. Figures 1 and 2 compare the amino acid sequences of all the pre-rbcS polypeptides published to date. Several conclusions can be drawn:

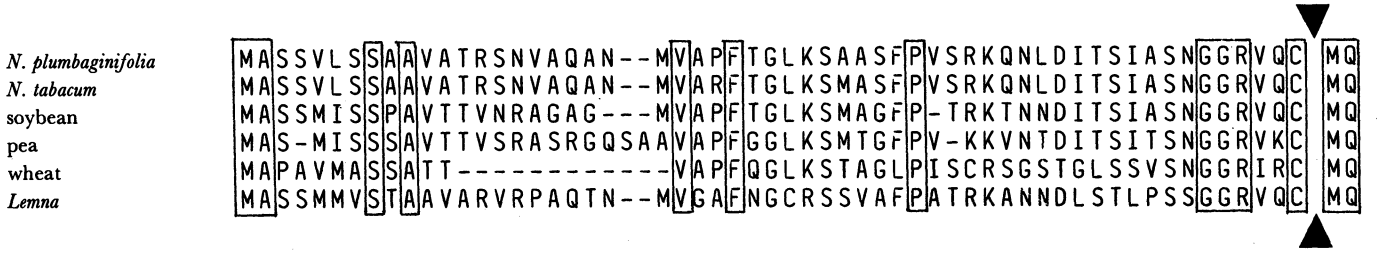

FIGURE 1. Comparison of the transit peptide of rbcS from *Nicotiana plumbaginifolia* (Poulsen *et al.*, 1986), *Nicotiana tabacum* (Mazur & Chui 1985), soybean (Berry-Lowe *et al.* 1982), pea (Coruzzi *et al.* 1983), wheat (Broglie *et al.* 1983) and *Lemna* (Wimpee 1984). Splicing site is indicated by an arrow. The first two amino acid residues of the mature polypeptide are also indicated. Residues identical in all species are boxed.

(1) There is less homology in the transit peptide than in the mature polypeptide. This implies that exon I of the rbcS genes evolved at a faster rate than exons II and III. An exception to this rule is the conservation of exon I and of the *Nicotiana* spp., which have their exon III split by a rapidly evolving intron (Poulsen *et al.* 1986). Many of the nucleotide changes in exon I result in conservative amino acid changes.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

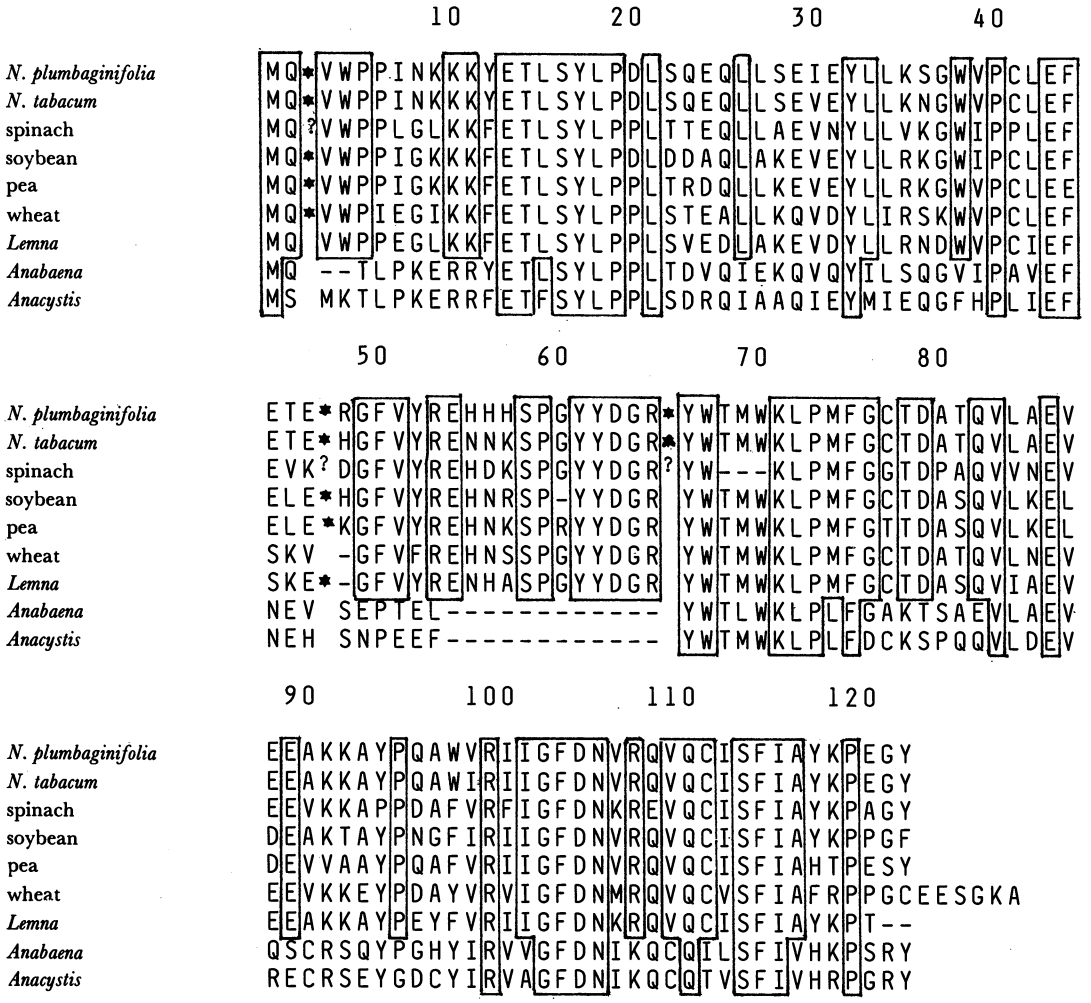

FIGURE 2. Comparison of the amino acid sequence of the mature rbcS from *Nicotiana plumbaginifolia* (Poulsen *et al.* 1986), *Nicotiana tabacum* (Mazur & Chui 1985), spinach (Martin 1979), soybean (Berry-Lowe *et al.* 1982), pea (Corruzi *et al.* 1983), wheat (Broglie *et al.* 1983), *Lemna* (Wimpee 1984), *Anabaena* (Nierzwicki-Bauer *et al.* 1984) and *Anacystis* (Shinozaki & Sugiura 1983). When present in the genomic clones, the introns are indicated by a star. No genomic sequence is available for spinach. Residues identical in all species (or all species but *Anabaena* and *Anacystis*) are boxed.

(2) The length of the transit peptide varies from 46 amino acids in wheat to 57 in pea and *Nicotiana*.

(3) The transit peptide has an overall basic character and contains many hydroxy- and amide-amino acids. We have proposed that the basic nature may be important for initial interaction with the chloroplast envelope which is negatively charged (Broglie *et al.* 1983). All pre-rbcS polypeptides have Cys-Met-Gln as a conserved processing site; this sequence results in the conservation of an SphI site in the nucleotide sequence located at the 3′ border of exon I.

(4) The length of the mature rbcS polypeptide varies from 120 amino acids in *Lemna* to 128 in wheat. The difference in length is caused by the lack of sequence homology at the carboxy-terminus.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(5) With the exception of wheat, different members of the rbcS gene families of individual plant species encode polypeptides of identical amino acid sequence within that species.

(6) Compared to higher plant rbcS polypeptides, two cyanobacterial rbcS polypeptides have a deletion of a dodecapeptide (from position 54 to position 65) (Shinozaki & Sugiura 1983; Nierzwicki-Bauer *et al.* 1984). This observation suggests that the dodecapeptide of the higher-plant rbcS polypeptide is probably not directly involved in holoenzyme assembly or catalytic function, but it could be at a site that interacts with the eukaryotic assembly protein.

In most higher plants, the rbcS polypeptide is encoded by a small multigene family ranging from 2 (Poulsen *et al.* 1986) to 12 members. The presence of pseudogenes has been reported in petunia (Dean *et al.* 1985). Many of the gene members are linked in the nuclear genome, either in tandem (Dean *et al.* 1985) or in inverted orientation (Cashmore 1983). For example, five of the ten rbcS genes in petunia are found within a 23-kilobase stretch of nuclear DNA (Dean *et al.* 1985) and the rbcS genes of peas are all localized on chromosome 5 (Polans *et al.* 1985). The clustering of rbcS genes suggests that they could have arisen by gene duplication.

Organ-specific and cell-type-specific expression

Since the rbcS genes encode a polypeptide subunit of a major photosynthetic enzyme, it is perhaps not surprising that in most higher plants, these genes are expressed only in plant organs that contain chloroplasts. Northern analyses with pea plants indicate that while the rbcS mRNA is a major transcript in green leaves, it is also present in substantial amounts in other photosynthetic organs, such as petals, pods and pea seeds. Depending on their age, stems contain 5–50% of the rbcS level in green leaves; on the other hand, roots contain less than 1% of the leaf rbcS mRNA level (Coruzzi *et al.* 1984). The different levels of rbcS mRNA in the various organs probably reflect the varying amounts of chloroplast-containing cells in these organs (Eckes *et al.* 1985).

Even in leaves, the rbcS genes are not expressed in all cell types. These genes are probably inactive in epidermal cells, but are active in guard cells and mesophyll cells (Zemel & Gepstein 1985), both of which contain ribulose 1,5-bisphosphate carboxylase. In C4 plants, the rbcS polypeptide is present in bundle sheath cells but not in mesophyll cells, and this differential expression is controlled at least at the level of steady-state mRNA (Broglie *et al.* 1984*b*). Together, these results provide evidence that the expression of rbcS genes can be regulated by cell-type-specific factors, as well as by light.

Light induction

In all angiosperms investigated to date, the expression of rbcS genes has been shown to be inducible by light (cf. Tobin & Silverthorne 1985). However, the extent of light induction varies considerably among species. To a large degree, the light induction ratio is dependent on the amount of rbcS mRNA accumulated in the dark, which in turn is likely to be related to the stage of etioplast development of the species in question (Thompson *et al.* 1983). Cereals such as barley (Apel & Kloppstech 1978) and wheat (B. Keith, unpublished data) contain substantial amounts of rbcS mRNA in etiolated leaves, and their mRNA level is increased only moderately upon exposure to light, resulting in low light induction ratios. Among the legumes, the light induction ratio of rbcS gene expression is higher in pea than in mung bean,

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

presumably correlated with a more mature developmental stage of mung bean plastids in etiolated tissues (Thompson *et al.* 1983).

In *Lemna* (Tobin 1981) as well as in pea (Thompson *et al.* 1983; Sasaki *et al.* 1984; Jenkins *et al.* 1983), the rbcS level in etiolated tissues can be elevated by a pulse of red light. The induction of rbcS gene expression by red light is reversible by far-red light providing evidence that the effect is mediated by the photomorphogenic pigment phytochrome. By the use of a 3′ gene-specific probe, we have shown that at least two members, 3A and 3C, of the pea rbcS gene family are regulated by phytochrome (Fluhr & Chua 1986). The phytochrome regulation is exercised primarily at the transcriptional level as shown by rbcS transcript run-off experiments with isolated nuclei (Silverthorne & Tobin 1984; Berry-Lowe *et al.* 1985).

Whereas etiolated pea plants display a clear-cut phytochrome effect, the photoresponses of rbcS genes in green plants appear to be more complicated. The rbcS mRNA is a major transcript in leaves of green pea plants (Coruzzi *et al.* 1984), but it decreases in abundance by a factor of 50 when the plants are kept in the dark for two days. In contrast to etiolated plants, the rbcS transcript level in the dark-adapted green plants is not affected by a red light pulse, but it can be induced by continuous white light (Jenkins *et al.* 1983). We have shown that the effective wavelength for the white light induction is in the blue region and that the enhancing effect of blue light can be abrogated by far-red light (Fluhr & Chua 1986). These results suggest that two photoreceptors, a putative blue-light receptor and phytochrome, collaborate in the regulation of rbcS genes in mature green leaves. A similar requirement for the cooperation between the two photoreceptors has also been reported for anthocyanin synthesis in *Sorghum vulgare* (Oelmuller & Mohr 1985).

The light-inducibility of the rbcS genes is strongly modulated by the physiological state of the cells. Stress conditions can override the effect of light on rbcS gene expression; for example, heat-shocked leaves show reduced rbcS gene expression even in the presence of light (Vierling & Key 1985). Also, in protoplasts freshly prepared from mesophyll cells of *Nicotiana sylvestris* the rbcS mRNA decreased to an undetectable level (Vernet *et al.* 1982); this result strongly suggests that the transcriptional programme of mesophyll cells can be altered by the procedure of protoplast preparation, and cautions against the use of mesophyll protoplasts for examination of transient expression of foreign rbcS genes.

Expression of rbcS genes in transformed calluses

The regulation of rbcS gene expression by light raises the question of the identification of cis-acting elements involved in the photoresponses. This question can be approached by investigating the effects of sequence modification of an rbcS gene on its light-inducibility, provided that a suitable assay system exists for the accurate interpretation of the mutant gene constructs. The availability of Ti-mediated gene transfer systems (Fraley *et al.* 1983) has opened the way for this type of investigation. In the first series of experiments, a pea rbcS gene (E9) that has approximately 1 kb of 5′ upstream sequence was transferred into petunia cells by an oncogenic Ti vector (Broglie *et al.* 1984*a*). A hybrid gene comprising the nopaline synthase (NOS) promoter fused to the coding sequence of bacterial neomycin phosphotransferase (nptII) was used as the selectable marker. Northern analysis showed that the inserted rbcS–E9 gene is expressed in light-grown, transformed petunia calluses at levels of 20–50 times higher than in dark-adapted calluses, whereas the covalently linked NOS–nptII gene is expressed equally

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

in both types of tissue. The pea rbcS–E9 transcript isolated from transformed petunia calluses has the authentic 5′ end, appears to be spliced accurately, and is polyadenylated at the correct site. Together, these results provide evidence that the regulatory and promoter sequences of the pea rbcS–E9 gene are correctly recognized by the nuclear transcriptional machinery of petunia cells to give light-inducibility of the foreign rbcS gene. Similar light-regulated expression has also been reported for another pea rbcS gene (SS3.4) (Herrera-Estrella *et al.* 1984) and a soybean rbcS gene (Facciotti *et al.* 1985) in tobacco calluses.

In transformed petunia calluses, the pea rbcS gene is expressed, not only at the transcript level, but also at the polypeptide level (Broglie *et al.* 1984*a*). The pea rbcS polypeptides are localized within petunia chloroplasts in association with petunia rbcL (large subunit) polypeptides to form hybrid ribulose 1,5-bisphosphate carboxylase holoenzyme.

The retention of light-inducible expression of the pea rbcS–E9 gene in petunia calluses justifies the use of this expression system for the identification of 5′ sequence elements needed for maximal expression and light-inducibility. To this end, we constructed a series of 5′ deletion mutants by Bal31 digestion. The mutants were transferred into petunia cells by Ti-plasmid vectors and their transcriptional properties in light- and dark-grown calluses were assessed by Northern blots, with the NOS-nptII mRNA as an internal control (Morelli *et al.* 1985). We found that the 5′ deletion of the rbcS–E9 from nucleotide −1052 to −437 (5′Δ-437) results in a 3- to 4-fold decrease in the rbcS mRNA relative to the NOS-nptII mRNA. Additional deletion of 85 nucleotides to position −352 (5′Δ-352) reduces the mRNA level further by a factor of 2 to 3. Low but detectable levels of rbcS mRNA are present in mutants 5′Δ-255, 5′Δ-96 and 5′Δ-87 and 5′Δ-35, respectively whereas mutant 5′Δ-13 is inactive. Mutant 5′Δ-107/-56, which has an internal deletion of 51 bp surrounding the CCAT box region, is twice as active as the wild type. All the active 5′ deletion mutants, as well as 5′Δ-107/-56, show light regulation, irrespective of their level of expression. Taken together, these results suggest that mutants which contain only 35 nucleotides in the 5′ upstream region are still responsive to light-stimulation. In another series of experiments, we have shown that the rbcS–E9 promoter fragment (−1052 to −2) is sufficient to confer light-regulated expression when joined to a test gene. On the other hand, a hybrid gene comprising a cauliflower mosaic virus 35S promoter fused to the coding sequence of pea rbcS–E9 is expressed equally in the light and dark; this result suggests that light has no effect on that rbcS–E9 transcript stability. From these results we conclude that a 33 bp sequence, between −35 and −2, is involved in the light-regulated expression of pea rbcS–E9. The 5′ upstream sequences from −352 to −1052 are needed for maximal expression of rbcS–E9 in petunia calluses, but whether this DNA segment is also important for light-inducibility remains to be tested.

Expression in transgenic plants

The use of transformed calluses as an expression system for foreign rbcS genes is subject to certain limitations. Since calluses are non-physiological tissues, we do not know to what extent the light-regulated expression of the rbcS genes reflects the physiological reactions in mesophyll cells. Furthermore, calluses transformed by oncogenic Ti-vectors contain high amounts of phytohormones, which may modify the photo-response of the inserted rbcS gene. Finally, transformed calluses are non-morphogenic, thus precluding investigations on organ-specific expression of the introduced genes. To obviate these potential problems, we repeated the gene

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

transfer experiments with a 'disarmed' Ti-vector, which allowed plant regeneration (Fraley *et al.* 1985).

The pea rbcS–E9 gene, containing 1 kb of 5′ upstream sequence, was transferred into both *Petunia hybrida* and *Nicotiana tabacum* (Nagy *et al.* 1985). We found that the gene was expressed at a higher level in transgenic petunia than in tobacco, and that it was light-inducible and organ-specific in both species. These results indicate that the introduced pea rbcS–E9 gene contains the requisite cis-acting elements for the regulated expression and that the gene regulatory signals are recognized by the transcriptional apparatus of other dicots. The rbcS–E9 mRNA levels vary over 50-fold among independent transgenic petunia plants; this result suggests that the expression of the introduced gene is sensitive to its chromosomal insertion site. In spite of this chromosomal position effect, the rbcS–E9 gene continues to be light-induced and expressed in the appropriate organs in all the clones examined. Therefore, the quantitative rather than the qualitative aspects of rbcS–E9 gene expression appear to be sensitive to the chromosomal location of the recipient genome.

In contrast to the results obtained when transformed calluses were used as an expression system (Morelli *et al.* 1985), deletion of the rbcS–E9 5′ upstream sequence from −1052 to −352 has no measurable effect on the mRNA level expressed in transgenic petunia leaves (Nagy *et al.* 1985). Moreover, the 5′Δ-352 mutant of rbcS–E9 still exhibits the same light-inducibility and organ-specific expression as the wild type. Analyses of other 5′ deletion mutants and chimaeric constructs are under way.

We have also transferred two additional members (3A and 3C) of the pea rbcS gene family into petunia and tobacco and shown that both genes retain their light-regulated and leaf-specific expression (Fluhr & Chua 1986). More importantly, the phytochrome-induced expression of the two genes can be recapitulated in etiolated leaves of transgenic plants. Current efforts in our laboratory are directed toward the identification of cis-acting elements that mediate the phytochrome response.

Conclusions and prospects

Molecular cloning and sequencing of genes coding for rbcS have provided detailed information on the primary structure of these genes. In all higher plants investigated so far, the rbcS genes are present as small multigene families with the number of introns per gene varying between 1 and 3. The presence of multiple genes for a single protein immediately raises the question of whether there is any difference in expression among the individual members. There are no dramatic differences in the pattern of organ-specificity among the genes, all of them being most highly expressed in the leaves and lower in the other green tissues (Coruzzi *et al.* 1984). The absolute level of expression, however, varies considerably; in light-grown pea leaves, the rbcS–3A gene is expressed at a level approximately 20 times higher than the rbcS–E9 gene (R. Fluhr, unpublished results). Experiments have not yet detected a qualitative difference of light response of these genes.

The *Agrobacterium*-mediated gene transfer system was used to localize the DNA sequences that confer regulated expression of the rbcS genes. Several pea genes have been transferred to tobacco and petunia by this method and shown to be correctly expressed. Three pea rbcS genes were transferred to petunia cells by means of a 'disarmed' T-DNA vector, and correct organ-specific expression was demonstrated in the regenerated (transgenic) plants (Nagy *et*

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

al. 1985; Fluhr & Chua 1986). This provides conclusive evidence that the tobacco and petunia cells are capable of faithfully recognizing the heterologous pea rbcS regulating sequences. By constructing genes that are altered with respect to the original (wild-type) rbcS gene, and transferring these mutant genes to the new host, the specific sequences involved in gene regulation can be identified. A 33-bp element involved in light-induced transcription has been identified by this method (Morelli *et al.* 1985). The approach of *in vitro* mutagenesis coupled with expression in transgenic plants has the potential to localize any other cis-acting DNA regulatory elements that are required for light-induced and organ-specific expression.

Our interest is to understand the complete sequence of events leading to the regulated expression of the rbcS genes. How does light turn on transcription? Does phytochrome interact directly with regulatory DNA elements, or is there a longer transduction chain? And what is the nature of the factors that induce transcription in leaves and stems, but not in roots? A careful analysis of all the rbcS regulatory DNA elements, in combination with biochemical and genetic characterization of trans-acting factors, will be needed to answer such questions.

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REFERENCES

Andrews, T. J. & Ballment, B. 1983 The function of the small subunits of ribulose bisphosphate carboxylase–oxygenase. *J. biol. Chem.* **258**, 7514–7518.

Apel, K. & Kloppstech, K. 1978 The plastid membranes of barley (*Hordeum vulgare*): Light-induced appearance of mRNA coding for the apoprotein of the light-harvesting chlorophyll *a*/*b*-protein. *Eur. J. Biochem.* **85**, 581–588.

Berry-Lowe, S. L., McKnight, T. D., Shah, D. M. & Meagher, R. B. 1982 The nucleotide sequence, expression and evolution of one member of a multigene family encoding the small subunit of ribulose-1,5-bisphosphate carboxylase in soybean. *J. molec. appl. Genet.* **1**, 483–498.

Berry-Lowe, S. L. & Meagher, R. B. 1985 Transcriptional regulation of a gene encoding the small subunit of ribulose-1,5-bisphosphate carboxylase in soybean tissue is linked to the phytochrome response. *Molec. Cell Biol.* **5**, 1910–1917.

Broglie, R., Coruzzi, G., Lamppa, G., Keith, B. & Chua, N.-H. 1983 Structural analysis of nuclear genes coding the precursor to the small subunit of wheat ribulose-1,5-bisphosphate carboxylase. *Biotechnology* **1**, 55–61.

Broglie, R., Coruzzi, G., Fraley, R. T., Rogers, S. G., Horsch, R. B. *et al.* 1984*a* Light-regulated expression of a pea ribulose-1,5-bisphosphate carboxylase small subunit gene in transformed plant cells. *Science, Wash.* **224**, 838–843.

Broglie, R. Coruzzi, G., Keith, B. & Chua, N.-H. 1984*b* Molecular biology of C_4 photosynthesis in *Zea mays*: differential localization of proteins and mRNAs in the two leaf cell types. *Pl. mol. Biol.* **3**, 431–444.

Cashmore, A. R. 1983 Nuclear genes encoding the small subunit of ribulose-1,5-bisphosphate carboxylase. In *Genetic engineering of plants* (ed. T. Kosuge, C. P. Meedith & A. Hollaender), pp. 29–38. New York: Plenum.

Coruzzi, G., Broglie, R., Cashmore, A. & Chua, N.-H. 1983 Nucleotide sequences of two pea cDNA clones encoding the small subunit of ribulose-1,5-bisphosphate carboxylase and the major chlorophyll *a*/*b*-binding thylakoid polypeptide. *J. biol. Chem.* **258**, 1399–1402.

Coruzzi, G., Broglie, R., Edwards, C. & Chua, N.-H. 1984 Tissue-specific and light-regulated expression of a pea nuclear gene encoding the small subunit of ribulose-1,5-bisphosphate carboxylase. *EMBO J.* **3**, 1671–1679.

Dean, C., van den Elzen, P., Tamaki, S., Dunsmier, P. & Bedbrook, J. 1985 Linkage and homology analysis divides the eight genes for the small subunit of petunia ribulose-1,5-bisphosphate carboxylase into three gene families. *Proc. natn. Acad. Sci U.S.A.* **82**, 4964–4968.

Eckes, P., Schell, J. & Willmitzer, L. 1985 Organ-specific expression of three leaf/stem specific cDNAs from potato is regulated by light and correlated with chloroplast development. *Molec. gen. Genet.* **199**, 216–224.

Ellis, R. J. 1981 Chloroplast proteins: Synthesis, transport and assembly. *A. Rev. Pl. Physiol.* **32**, 111–137.

Facciotti, D., O'Neal, J. K., Lee, S. & Shewmaker, C. K. 1985 Light-inducible expression of a chimaeric gene in soybean tissue transformed with *Agrobacterium*. *Biotechnology* **3**, 241–246.

Fluhr, R. & Chua, N.-H. 1986 Developmental regulation of two genes encoding ribulose-bisphosphate carboxylase small subunit in pea and transgenic petunia plants: Phytochrome response and blue-light induction. *Proc. natn. Acad. Sci. U.S.A.* **83**, 2358–2362.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Fraley, R., Rogers, S., Horsch, R. Sanders, P., Flick, J. S. *et al.* 1983 Expression of bacterial genes in plant cells. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4803–4807.

Fraley, R. T., Rogers, S. G., Horsch, R. B., Eichhotz, D. A., Flick, J. S. *et al.* 1985 The SEV systems: A new disarmed Ti plasmid vector system for plant transformation. *Biotechnology* **3**, 629–635.

Gatenby, A. A., Van der Vies, S. M. & Bradley, D. 1985 Assembly in *E. coli* of a functional multi-subunit ribulose bisphosphate carboxylase from a blue-green alga. *Nature, Lond.* **314**, 617–620.

Herrera-Estrella, L., Van den Broeck, G., Maenhaut, R., van Montagu, M, Schell, J. *et al.* 1984 Light-inducible and chloroplast-associated expression of a chimaeric gene introduced into *Nicotiana tabacum* using a Ti plasmid vector. *Nature, Lond.* **310**, 115–120,

Hollis, G. F., Hieter, P. A., Mcbride, O. W., Swan, D. & Leder, P. 1982 Processed genes: A dispersed human immunoglobulin gene bearing evidence of RNA-type processing. *Nature, Lond.* **196**, 321–325.

Jenkins, G. I., Hartley, M. R. & Bennett, J. 1983 Photoregulation of chloroplast development: transcriptional, translational, and post-translational controls? *Phil. Trans. R. Soc. Lond.* B **303**, 419–431.

Martin, P. G. 1979 Amino acid sequence of the small subunit of ribulose-1,5-bisphosphate carboxylase from spinach. *Aust. J. Pl. Physiol.* **6**, 401–408.

Mazur, B. J. & Chui, C-F. 1985 Sequence of a genomic DNA clone for the small subunit of ribulose bisphosphate carboxylase–oxygenase from tobacco. *Nucl. Acids Res.* **13**, 2373–2386.

Morelli, G., Nagy, F., Fraley, R. T., Rogers, S. G. & Chua, N.-H. 1985 A short conserved sequence is involved in the light-inducibility of a gene encoding ribulose-1,5-bisphosphate carboxylase small subunit of pea. *Nature, Lond.* **315**, 200–204.

Nagy, F., Morelli, G. Fraley, R. T., Rogers, S. G. & Chua, N.-H. 1985 Photoregulated expression of a pea rbcS gene in leaves of transgenic plants. *EMBO J.* **12**, 3063–3068.

Nierzwicki-Bauer, S. A., Curtis, S. E. & Haselkorn, R. 1984 Cotranscription of genes encoding the small and large subunits of ribulose-1,5-bisphosphate carboxylase in the cyanobacterium *Anabaena* 7120. *Proc. natn. Acad. Sci. U.S.A.* **81**, 5961–5965.

Oelmuller, R. & Mohr, H. 1985 Mode of coaction between blue/UV light and light absorbed by phytochrome in light-mediated anthocyanin formation in the milo (*Sorghum vulgare* Pers.) seedling. *Proc. natn. Acad. Sci. U.S.A.* **82**, 6124–6128.

Polans, N. O., Weeden, N. F. & Thompson, W. F. 1985 Inheritance, organization and mapping of rbcS and Cab multigene families in pea. *Proc. natn. Acad. Sci. U.S.A.* **82**, 5083–5087.

Poulsen, C., Fluhr, R., Kauffman, J. M., Boutry, M. & Chua, N.-H. 1986 (In preparation.)

Sasaki, Y., Tomoda, Y. & Kamikubo, T. 1984 Light regulates the gene expression of ribulose bisphosphate carboxylase at the levels of transcription and gene dosage in greening pea leaves. *FEBS Lett.* **173**, 31–35.

Shinozaki, K. & Sugiura, M. 1983 The gene for the subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase is located close to the gene for the large subunit in the cyanobacterium *Anacystis nidulans* 6301. *Nucl. Acids Res.* **11**, 6957–6964.

Silverthorne, J. & Tobin, E. M. 1984 Demonstration of transcriptional regulation of specific genes by phytochrome action. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1112–1116.

Thompson, W. F., Everett, M. N., Polans, N. O., Jorgensen, R. A. & Palmer, J. D. 1983 Phytochrome control of RNA levels in developing pea and mung-bean leaves. *Planta* **158**, 487–500.

Tobin, E. M. 1981 Phytochrome-mediated regulation of messenger RNAs for the small subunit of ribulose 1,5-bisphosphate carboxylase and the light-harvesting chlorophyll *a*/*b*-protein in *Lemna gibba*. *Pl. molec. Biol.* **1**, 35–51.

Tobin, E. M. & Silverthorne, J. 1985 Light regulation of gene expression in higher plants. *A. Rev. Pl. Physiol.* **36**, 569–593.

Vernet, T., Fleck, J., Durr, A., Fritsch. C., Pinck, M. & Hirth, L. 1982 Expression of the gene coding for the small subunit of ribulose bisphosphate carboxylase during differentiation of tobacco plant protoplasts. *Eur. J. Biochem.* **126**, 4489–4494.

Vierling, E. & Key, J. L. 1985 Ribulose-1,5-bisphosphate carboxylase synthesis during heat shock. *Pl. Physiol.* **78**, 155–162.

Wimpee, C. F. 1984 Organization and expression of light-regulated genes in *Lemna gibba* L. G-3. Ph.D. thesis, University of California, Los Angeles.

Zemel, E. & Gepstein, S. 1985 Immunological evidence for the presence of ribulose-bisphosphate carboxylase in guard cell chloroplasts. *Pl. Physiol.* **78**, 586–590.

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