Photosynthesis-Associated Gene Families: Differences in Response to Tissue-Specific and Environmental Factors

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The endogenous small subunit of the ribulose-1,5-bisphosphate carboxylase gene *rbcS* and the light-harvesting chlorophyll a/b–binding protein gene (*LHCP*) of pea are expressed in a light-inducible manner and are active mainly in green chloroplast–containing tissue. Chimeric genes under control of the 5′-flanking sequences of the *rbcS ss*3.6 or *LHCP AB*80 genes from pea were used to study the factors relating to the tissue-specific and light-inducible expression of these nuclear-encoded genes in transgenic tobacco plants. The results show that plastid development plays a crucial role in the activation of expression of these chimeric genes. Particular members of each of the above gene families respond differently to tissue-specific and environmental factors. Furthermore, the light-inducible expression directed by the 5′-flanking sequence of *ss*3.6 *rbcS* gene is not exclusively mediated by phytochrome, but probably is controlled in large part by another photoreceptor.

THE ESTABLISHMENT OF REPRODUCIBLE METHODS TO INtroduce DNA into plant cells has opened the way to study the regulation of plant genes at the molecular level. Analyses of the regulation of plant genes have so far focused mainly on two gene families: (i) those encoding the small subunit of the ribulose-1,5-bisphosphate carboxylase (*rbcS*), and (ii) those encoding the light-harvesting chlorophyll a/b–binding proteins (*LHCP*). (This protein binds both chlorophyll a and chlorophyll b.) Both polypeptides are encoded in the nuclear genome as small multigene families in higher plants (*1, 2*). Although the *LHCP* and *rbcS* genes are encoded by nuclear DNA, their gene products are translocated into and functional in the chloroplasts (*3*). The LHCP's bind chlorophyll and become integrated in the thylakoid membranes where they participate in harvesting energy from light and transferring the resulting excitation energy to photosystems I and II (*4*). In contrast, the small subunit of the ribulose-1,5-bisphosphate carboxylase (SS) combines with the chloroplast-encoded large subunit of ribulose-1,5-bisphosphate carboxylase to form the active enzyme and remains within the chloroplast stroma (*5*). Transcripts of both genes are present in specific tissues, and are most abundant in photosynthetic tissue such as leaf and pericarps, but they are not present in roots (*6*). The expression of the *LHCP* and *rbsS* genes has been correlated with the presence of chloroplasts or etioplasts. Study of these genes therefore should help to elucidate how the process of photosynthesis is regulated.

The *LHCP* genes are light-inducible in all plants examined up until now—for example, *Lemna*, barley, pea, maize, and petunia (*7*). The *rbcS* genes are inducible by light in *Lemna*, pea, tobacco, and soybean, but are only marginally influenced by light in barley and mung bean (*7*).

In addition, different components of white light have been shown to affect both sets of genes. Plants exposed to red light after growth in darkness have greatly increased levels of messenger RNA (mRNA) for *rbcS* and *LHCP* (*8*). This effect can be reversed if a pulse of far-red light is given immediately after red light treatment. This stimulation by red light and its reversion by far-red light is the conventional demonstration for the involvement of phytochrome as a photoreceptor.

The possibility of introducing and expressing foreign genes in plant cells and following this expression by use of specific probes or by constructing chimeric genes containing easily detectable markers has resulted in rapid progress in the understanding of the processes of regulation of eukaryotic genes and identification of the responsible DNA sequences. In plants, these types of studies have shown that the 5′-flanking sequences of *rbcS* and *LHCP* genes isolated from pea are sufficient to direct light-regulated and tissue-specific expression of chimeric gene constructions (*9–11*).

We now present further data concerning the expression in transgenic plants of chimeric constructions involving the 5′-flanking sequences of the *rbcS* gene *ss*3.6 (*1*) and the *LHCP* gene *AB*80 (*12*), both of which have been isolated from pea. We report further on the tissue-specific expression of these constructs and demonstrate by herbicide treatment that the tissue specificity patterns are probably correlated to the developmental or metabolic stage of plastids. In addition, the effects of different light regimes on the regulation of the chimeric constructs were also examined.

Organ-specific expression. The fusions of specific promoters to particular genes permit us to determine whether the expression directed by these specific 5′-flanking sequences of the *LHCP* and *rbcS* gene families give a similar pattern of expression as the complete gene families and to compare the expression patterns of two different photosynthesis-associated genes. We have focused on the tissue-specific pattern of expression directed by the 5′-flanking sequence of the *ss*3.6 and *AB*80 genes in transgenic plants.

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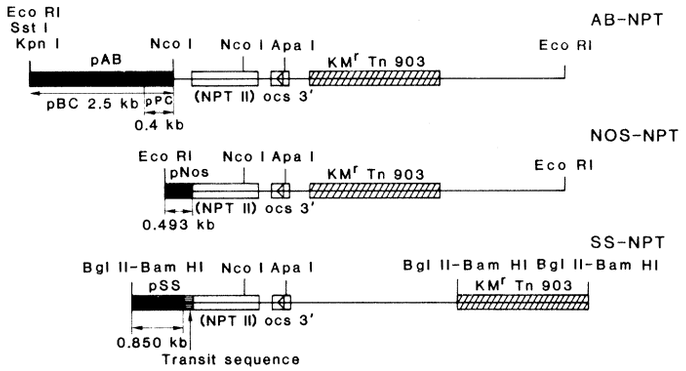


Fig. 1. The AB-NPT(II), SS-NPT(II), and NOS-NPT(II) chimeric gene constructions. The SS-NPT(II) gene contains 930 base pairs of the *ss*3.6 5′-flanking sequences, and the AB-NPT(II) is under control of either 2.5 or 0.4 kb of the 5′-flanking sequences of the *AB*80 gene. The details of the construction of these chimeric genes have been described (*9, 25*). The deduced sequence of the transcripts produced by these genes share 1600 identical nucleotides, while the SS-NPT(II) and the AB-NPT(II) incorporate 180 and 90 extra nucleotides at the 5′ end with respect to the NOS-NPT(II) transcript. No homology is found between the extra nucleotides present in the AB-NPT(II) and SS-NPT(II) transcripts.

We used chimeric gene constructions consisting of 5′-flanking sequences of the *LHCP* gene *AB*80 and the *rbcS* gene *ss*3.6 fused to the neomycin phosphotransferase II [NPT(II)] gene from the transposon Tn5 [AB-NPT(II) and SS-NPT(II), respectively]. A third construct consisting of the constitutively expressed nopaline synthase (NOS) promoter fused to the NPT(II) gene [NOS-NPT(II)] was used as a control (Fig. 1).

Transformed *Nicotiana tabacum* shoots containing the chimeric constructs were obtained by *Agrobacterium*-mediated transformation of leaf disk cultures, regeneration, and subsequent growth of induced shoots to full maturity. To determine the tissue-specific pattern of expression directed by the *ss*3.6 and the *AB*80 promoter, samples were taken from leaves, stem, roots, sepals, stigmas, and petals of several transgenic plants containing AB-NPT(II) or SS-NPT(II) constructions, and the level of NPT(II) activity produced by these chimeric constructs in each tissue was determined from cellular extracts. Control plants containing the NOS-NPT(II) construct showed similar levels of expression in all tissues tested.

The results from five regenerated plants containing the SS-NPT(II) construct (rSSa to rSSe) and five regenerated plants containing either 2.5 kilobases (kb) of *AB*80 5′-flanking sequences (rABacde) or 0.4 kb of *AB*80 5′-flanking sequences (rABb) are shown in Fig. 2. The rAB plants show high levels of NPT(II) expression in leaves and stem and low levels in sepal tissue after overexposure of the autoradiograms (Fig. 2, rABa). However, even after overexposure of the autoradiogram no NPT(II) activity could be detected in either roots or petals and only one rAB (rABd) plant shows NPT(II) activity in stigmas. In addition, with the exception of a lower level of expression in the stem relative to that present in leaves, rABb containing only 0.4 kb of the 5′-flanking sequence of the *AB*80 gene shows the same tissue-specific pattern of expression as the plants containing the 2.5-kb AB 5′-flanking sequence. As can be observed, in transgenic plants rSSa to rSSd (Fig. 2) the SS-NPT(II) gene is predominantly expressed in leaves, sepals, and stigmas. There is also a low but detectable level of expression in stems after longer exposures of the autoradiograms to that shown in Fig. 2. No activity was detected in roots or petals, even after overexposure of the autoradiogram. This shows that the chimeric genes are only expressed in tissue containing mature chloroplasts and not in other tissues such as petals or roots. In our tests on anther and seed tissue we did not detect any activity in these tissues for either rAB or rSSU plants.

Although there is a considerable variation in the quantitative level of expression probably due either to copy number or position effects, the tissue-specific pattern of expression is rather consistent for most of the rAB or rSSU transgenic plants analyzed to date. However, we have found that one rSSU plant and one rAB plant show a different pattern of expression from those of the other plants. The plant rSSe has low levels of expression in leaves, stem, and sepals and a very high level of expression in petals (Fig. 2B). In petal tissue no NPT(II) expression has been detected in any other transgenic plant tested so far. Furthermore, the rABe plant NPT(II) activity is higher in sepals than in leaves and is very low in stem. In the more normal situation observed for the remaining plants, the *AB*80 promoter sequence directs high levels of NPT(II) expression in leaves and stem, and a much lower level in sepals.

Other studies on transgenic plants have shown that quantitatively expression of genes transferred into plants is greatly influenced by

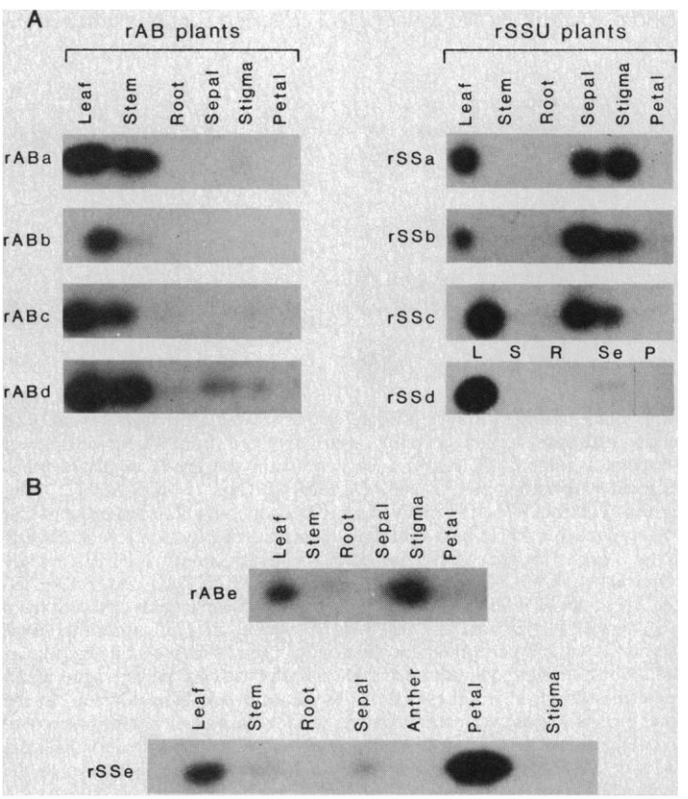


Fig. 2. Comparison of NPT(II) activity in different organs of transformed plants. Plasmids containing chimeric constructs were conjugated (*26*) from *Escherichia coli* to an *Agrobacterium* strain containing the nononcogenic Ti plasmid derivative pGV3850 (*27*), and exconjugants containing the chimeric genes between T-DNA borders were used to infect SR1 tobacco leaf disks (*28*) or protoplasts (*29*). Plants were regenerated from these cultures, and grown in a plant growth chamber in Vermiculite supplemented by commercial plant food (Substral) under conditions of 16 hours light and 8 hours darkness. Extracts were taken from leaf, stem, root, sepal, stigma, and petal tissue of plants transformed with either the AB-NPT(II) or the SS-NPT(II) construct rABa-e, and rSSa-e, respectively, and the NPT(II) activity in samples containing 100 μg of proteins was determined by the in situ detection method on nondenaturing polyacrylamide gels as described (*25, 30*). (A) Left column shows the results of four rAB plants, and the right column shows results of four rSS plants. (B) one rAB plant (rABe) and one RSS plant (rSSe) show different patterns of activity in comparison to the plants in part (A); L, leaf; S, stem; R, rod; Se, sepal; P, petal; rSSd, stigma not tested (only the relevant NPT(II) bands are shown).

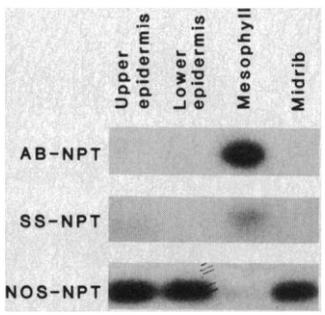
the site of insertion within the plant genome (*13*). Our results show that not only the quantitative level of expression but also the tissue-specific pattern of expression can be greatly altered in transgenic plants. These effects could be explained by "enhancer-like" elements [recently reported in plants (*14*)] which can affect gene transcription over distances of several kilobases. In these experiments, however, such effects may be weakened by the pBR322 and Ti plasmid sequences flanking the chimeric gene within the transferred DNA (T-DNA) of *Agrobacterium*. Perhaps if the foreign gene under study was located closer to a T-DNA border a greater number of position effects would be detected.

Cell type–specific gene expression. The above results show that the SS-NPT(II) and AB-NPT(II) genes are expressed in an organ-specific manner. However, since expression of the *LHCP* and *rbcS* genes has been correlated with the presence of chloroplasts (*15, 16*), it is conceivable that regulation may be specific not only to individual organs, but even more specifically to different cell types within an organ. Therefore, we extended our analysis to some of the tissues composing a leaf.

In order to determine whether the AB-NPT(II) and SS-NPT(II) constructions are expressed only in some specific tissues of the leaf, we determined the level of NPT(II) activity present in the upper epidermis, lower epidermis, mesophyll, and midrib tissues of rAB and rSS transgenic plants and compared them with those obtained from the same leaf tissues of NOS-NPT(II) transgenic plants (*17*).

The NOS-NPT(II) genes direct similar levels of expression in the upper and lower epidermis and midrib tissue, and to a lower extent in mesophyll tissue (Fig. 3). In contrast, both the AB-NPT(II) and the SS-NPT(II) genes direct high levels of expression in mesophyll cells and only very low levels of expression in lower epidermis and midrib tissues. The low NPT(II) activity observed in the lower epidermis is most likely due either to a high level of expression in the chloroplast-containing guard cell of the stomata or to contaminating mesophyll cells in the tissue extracts.

These results show that the AB-NPT(II) and SS-NPT(II) genes are expressed in a particular type within an organ, namely the photosynthetically active mesophyll cells, and confirms other observations that expression of these genes is correlated with the presence of mature chloroplasts.



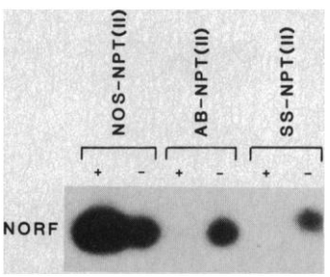


Fig. 3 (left). Comparison of NPT(II) activity in the different cell types of an individual plant organ. Extracts were obtained from upper and lower epidermis, mesophyll, and midrib tissue from the leaves of plants transformed with either the AB-NPT(II), SS-NPT(II), or NOS-NPT(II) constructs, and the NPT(II) activity in samples containing 50 μg of proteins was determined by the in situ detection method (*25, 30*) (see legend to Fig. 2). Fig. 4 (right). Effect of herbicide treatment on NPT(II) activity directed by AB-NPT(II), SS-NPT(II), and NOS-NPT(II) constructs. AB-NPT(II), SS-NPT(II), and NOS-NPT(II)-transformed seeds obtained from regenerated plants were left at 4°C for 2 weeks, after this time they were sterilized with 75 percent ethanol and hypochlorite, and were then germinated on half-strength Murashige and Skoog salts with 0.8 percent agar, and 1 percent sucrose, in the presence or absence of 1 μ*M* Norfluorazon. In the presence of sucrose such seedlings can survive for several weeks. Extracts of seedlings containing 25 μg of proteins were subsequently tested for NPT(II) activity (*25, 30*) (see legend to Fig. 2). NPT(II) activity is shown in the presence (+) or absence (−) of the herbicide (NORF).

Effect of the developmental state of chloroplasts on the expression of chimeric constructs. The tissue and cell type specificity analyses suggest that the chimeric constructs are only expressed in green chloroplast–containing tissue. However, observations that roots or callus tissue stimulated by cytokinins to become green do show activity suggest that tissues that are not green have the potential to express the *rbcS* and *LHCP* genes, but that this expression depends on certain stage of plastid development (*18–20*). In addition, in leaves of mutants blocked in carotene biosynthesis where plastid development is arrested in stages before the formation of mature chloroplasts, no detectable *LHCP* messenger RNA (mRNA) was found (*18–20*). Similar results were obtained when *LHCP* mRNA was measured in seedlings treated with Norfluorazon, a herbicide (*18–20*). This herbicide blocks carotenoid biosynthesis, thus also arresting plastid development and indirectly resulting in photooxidation of chlorophyll, producing completely white, although otherwise normal seedlings when grown in sucrose-containing medium. In order to determine whether the expression directed by the *AB*80 and *ss*3.6 5′-flanking regions are directly correlated to the presence of active chloroplasts or to other factors specific to leaves, we obtained white carotenoid-deficient seedlings by germinating transformed seeds in 10 μ*M* Norfluorazon and assessed the influence of carotenoid deficiency on the expression of SS-NPT(II) and AB-NPT(II) genes.

SS-NPT, AB-NPT, and NOS-NPT–transformed seeds were germinated on Murashige and Skoog medium with 1 percent sucrose; control samples were grown in the absence of herbicide and test samples were grown in media containing 10 μ*M* Norfluorazon. After 2 weeks, seedlings were harvested and tested for NPT(II) activity. The NPT(II) activities present in white, herbicide-treated seedlings and green, normal seedlings were compared (Fig. 4). The level of NPT(II) activity produced by the NOS-NPT(II) gene was not affected, and perhaps was increased when the seedlings were treated with the herbicide. In contrast, no expression could be detected for both the SS-NPT(II) and the AB-NPT(II) chimeric genes in herbicide-treated seedlings. Since similar transcripts and proteins are produced by all three genes, our results suggest that both the AB-NPT(II) and the SS-NPT(II) genes are not transcribed in leaves of herbicide-treated seedlings grown under normal light conditions.

To correlate plastid development with the expression of the AB-NPT(II) and SS-NPT(II), we examined seedlings germinated and grown for 4 to 6 weeks in the presence of Norfluorazon, then removed them to media without herbicide, and after periods of 0, 1, 3, and 10 days the NPT(II) activity was determined and the state of plastid development was observed by electron microscopy. Considerable NPT(II) activity was present in seedlings grown for 3 days in herbicide-free medium, even though no obvious greening could be observed at that time.

The stage of plastid development in herbicide-treated leaf cells after 0, 3, and 10 days of growth in herbicide-free medium is shown in Fig. 5. At day 0 (continuous growth in presence of Norfluorazon) plastids are irregular, many of them quite small and almost without internal organization. At day 3, the plastids have a more regular and typical "lens shape," no lamellar bodies are yet present, but a large number of vesicles are present and forming at the plastid periphery which later aggregate into the prolamellar bodies. After ten or more days, seedlings start to green and plastids have reached the stage of mature chloroplasts.

Since up to 50 percent of the NPT(II) (*21*) normally found in nontreated seedlings was already present 3 days after treated seedlings were released from the herbicide, this demonstrates that

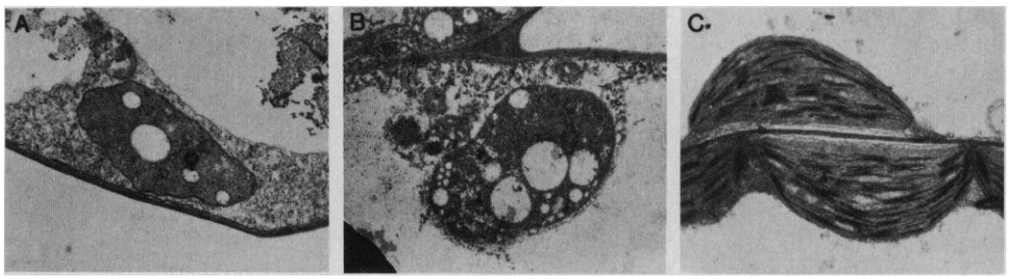


Fig. 5. Plastids of leaf cells after release from herbicide treatment. Electron micrography of plastids present in leaf cells following 0 (A), 3 (B), or 10 (C) days of growth of Norfluorazon-treated seedlings in the absence of the herbicide.

mature chloroplasts are not essential to trigger the expression of photosynthetic genes as represented by the AB-NPT(II) and SS-NPT(II) chimeric constructs. This experiment also suggests that metabolic or developmental pathways in very early stages of development between proplastid and chloroplast are sufficient for the expression of these genes.

Effect of different light regimes on expression of chimeric constructs. To determine whether the light-inducible response of the 5′-flanking sequences of the *rbcS* and *LHCP* genes is mediated by phytochrome action, we examined the effect of red and far-red light on the expression of the two representative chimeric genes. F1 seedlings obtained from transgenic plants containing the chimeric constructs in their genome were exposed to red and red followed by far-red light treatments, and the level of NPT(II) activity was determined for each light condition.

To this end, seeds obtained from rSS and rAB plants (previously tested to show a 75 percent transfer of the marker to the F1 generation) and from NOS-NPT transformed plants were germinated and grown in darkness on Murashige and Skoog medium with 1 percent sucrose. Control samples were kept either in continuous darkness or transferred to normal light conditions (16 hours light, 8 hours dark), and samples of dark-grown seedlings were exposed (i) to red light treatment three times daily for 10 minutes for 3 days, or (ii) to 10 minutes of red light, immediately followed by 10 minutes of far-red light, three times daily for 3 days. Two hours after the final red or far-red light treatment the seedlings were harvested and assayed for NPT(II) activity. The NOS-NPT(II) construction directs the synthesis of similar levels of NPT(II) activity under all light conditions (Fig. 6), except in the far-red light-treated sample which shows a higher activity. Whether this is a specific effect of far-red light on the expression of the *nos* promoter or it is a general increase in relative abundance of transcripts from constitutively expressed genes, remains to be determined. The AB-NPT construct is expressed when the seedlings are treated with white light, but not in darkness; it is also expressed in red light conditions, to a level similar to that obtained by white light treatment, and this expression is drastically reduced although not completely when red light is followed immediately by far-red light. This is consistent with the expectation that this gene is regulated by phytochrome and that the sequences mediating this response reside within the 5′-flanking sequence of the *AB*80 gene.

In contrast, the SS-NPT(II) construct is expressed when seedlings are grown in white light but, surprisingly, no expression could be detected under red or red and far-red conditions. This latter result was unexpected since these light conditions induce the synthesis of the endogenous small subunit polypeptides to normal levels. Thus, although the 5′-flanking region of the *ss*3.6 gene directs light-inducible expression of chimeric genes, this expression either requires red light fluences much higher than those normally sufficient to have full induction of the *rbcS* gene families or this expression is mainly mediated by a photoreceptor other than phytochrome and not completely activated by red light.

While in many systems the overall expression of the *rbcS* gene family is known to be under phytochrome control, it has been shown recently that certain members of the pea *rbcS* gene family only have a low fluence response to red light but are found to be greatly stimulated by blue light conditions (*22*). Initial experiments suggest that the expression mediated by the *ss*3.6 5′-flanking sequence is markedly increased by treatment of etiolated seedlings with blue light (*23*).

Implications of regulation by tissue-specific and environmental factors. Our results presented here demonstrate by means of chimeric gene constructs several of the factors affecting the regulation and expression of plant genes. One important feature is the tissue-specific expression of the genes. The SS-NPT(II) construct is expressed in all green tissues: leaves, stem, sepals, and stigma (a green structure in tobacco); however, levels of expression vary dramatically between the different tissues. These results agree qualitatively with the results of a previous study carried out on an individual *rbcS* gene (*6*). In that study (*6*), the *rbcS* gene, *pPS*2.4, also shows high expression in leaf, lower expression in stem, and no expression in petals or roots, although quantitatively the levels of expression in each tissue varies greatly from those presented above. In the same study the level of transcripts for the whole *rbcS* gene family was determined; this demonstrated that at least some members of the *rbcS* gene family are expressed in petal tissue. These data (*6*), in addition to the data presented here, suggest that the members of the *rbcS* gene family may be differentially regulated: some are expressed in certain tissues, such as petals, whereas others, like the *ss*3.6 and the *pPS*2.4, are not expressed in these tissues.

In contrast to the SS-NPT(II) construct, the AB-NPT(II) construct, although including sequences from a gene (*AB*80) whose product is also active in chloroplasts and participates in photosyn-

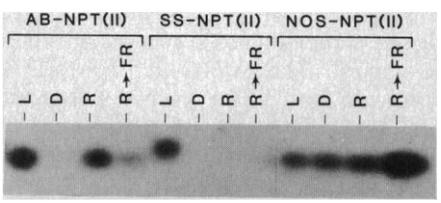


Fig. 6. Effect of different light regimes on the level of NPT(II) activity directed by either the AB, SS, or NOS 5′-flanking sequences. AB-NPT(II), SS-NPT(II), or NOS-NPT(II)-transformed seeds were germinated in darkness (legend to Fig. 4). Ten days after germination, seedlings were subsequently exposed to normal white light conditions (L), continuous darkness (D), or to a pulse of red light (R) three times daily for 3 days; red-fluorescent lamps (TL-IS, Philips) and a red filter (Rohm and Haas No501) were used. The fluence rate was 16.2 μmol m^{-2} sec^{-1}; the maximum wavelength was 650 nm. Or they were exposed to red light with a subsequent pulse of far-red light (R → FR) three times daily for 3 days with an Osram Lunistrons lamp filters through a 10-cm layer of H_2O (FRF-700 filter, Westlake Plastic Company). The fluence rate was 10.9 μmol m^{-2} sec^{-1} and the wavelength was greater than 700 nm. NPT(II) activity (*25, 30*) is shown under the different light conditions as described in the legend to Fig. 2.

thesis, shows a quite different pattern of expression. Again, expression occurs only in phytosynthetically active tissue, although quantitatively the levels of expression in comparison to the SS-NPT(II) construct vary greatly. The most striking difference is that with one exception no AB-NPT(II) activity could be detected in stigma tissue, whereas the SS-NPT(II) construct was expressed strongly in this tissue.

Our detailed studies involving different cell types within a single plant organ show that, the SS-NPT(II) and AB-NTP(II) constructs are only expressed strongly in mesophyll tissue, demonstrating a correlation in expression with the presence of chloroplasts. However, the different expression patterns of the SS-NPT(II) and AB-NPT(II) genes and of the individual members of the *rbcS* gene family suggest that in addition to light and the presence of developed plastids, other tissue-specific factors are involved in the regulation of these genes.

Experiments with the herbicide Norfluorazon confirm the role of plastids in the expression of chimeric genes consisting of sequences from nuclear genes associated with photosynthesis.

Since plastid development in carotenoid-deficient plants is arrested at an early stage (*18*), it is unclear whether carotenoid pigments or other factors related to chloroplast development are required for the light-inducible transcription of the *LHCP* and *rbcS* gene families. Nevertheless, our data and those of others (*18*) demonstrate that either the developmental stage or metabolic state of plastids (carotenoid synthesis occurs within the plastids) plays a crucial role in the regulation of expression of nuclear genes involved in photosynthesis. It is even possible to postulate that nuclear and plastid gene expression is coordinated to such an extent that not only nuclear gene products enter the chloroplast but that plastids also produce factors which enter and affect the regulation of nuclear genes.

The importance of plastid factors on the expression of photosynthetic genes is highlighted by the fact that herbicide-treated plants undergo other light-inducible phytochrome-mediated effects, such as hypocotyl elongation and anthocyanin accumulation, indicating that the whole light-inducible apparatus is probably present in herbicide-treated plants. This and the lack of expression of AB-NPT(II) and SS-NPT(II) constructs in plant tissues exposed to light, suggest that light alone is insufficient to stimulate expression of the chimeric constructs.

However, although light is insufficient by itself, it is also necessary for the expression of *LHCP* and *rbcS* genes. The treatment of seedlings to different light regimes suggests that the expression of the AB-NPT(II) chimeric construct, like endogenous *LHCP* genes, is under the control of phytochrome in so far as it is stimulated by red light and repressed by far-red light, and therefore, that the 2.5-kb 5′-flanking sequence of the *AB*80 gene is sufficient to confer this phytochrome control. The incomplete repression of the AB-NPT(II) construct in far-red light conditions is probably due to a very low fluence induction effect that affects the expression of endogenous *LHCP* genes (*24*). The lack of expression of the SS-NPT(II) chimeric gene in red light-treated seedlings suggests that phytochrome is not the major photoreceptor involved in the light inducibility of this particular member of the pea *rbcS* gene family, in contrast to other members of this gene family that are clearly inducible by phytochrome action (*22*). Some data indicates that a blue light photoreceptor may be involved instead (*23*).

Since the transcripts produced by all three chimeric constructs, AB-NPT(II), SSU-NPT(II), and NOS-NPT(II), are almost identical, it is unlikely that the observed differences in expression are due to differences in the translational capacity or stability of the mRNA, a strong indication that the observed differences are due to regulation at the level of transcription.

Our results show that although these two photosynthetic genes show similarities in their overall regulatory patterns, detailed analyses reveal a complex array of regulatory factors that distinguishes the two genes, including different responses to light conditions and very fine tissue-specific differences. These results involving only two individual genes, provide the first steps toward unraveling an increasingly intricate pattern of regulation and to ultimately determining which DNA sequences are involved in the various aspects of this regulation.

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