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# Cloning and characterization of a chalcone synthase gene from mustard and its light-dependent expression

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## Abstract

Genomic DNA from mustard was cloned in EMBL4 and screened for chalcone synthase (CHS) genes using a heterologous cDNA probe from parsley. Two clones which hybridized with the parsley cDNA probe were isolated. They showed different restriction patterns. One clone was sequenced and identified as a CHS gene by sequence comparison with published CHS sequences. The sequence of the coding region is 1188 bp, and encodes a protein of 43 kDa. The startpoint of transcription was determined by primer extension. The sequence of 0.9 kbp at the 5′ end of the transcription start and part of the noncoding 3′ of this gene were also determined. The coding sequence is interrupted by a single intron of 523 bp. The coding and the noncoding 5′ sequence of this gene was compared with CHS genes from other species. A very high homology was found with the *Arabidopsis* CHS coding region. A sequence motif (CACGTGT) which is present in most *rbcS* and all CHS upstream regions, and which specifically binds a protein factor from plant nuclear extracts, is also present in the upstream region of the mustard CHS gene.

Measurements of CHS transcript levels show that phytochrome controls expression of this gene in cotyledons of mustard seedlings; however, blue/UV-light photoreceptors control expression in later stages of development.

## Introduction

Chalcone synthase (CHS) is the key enzyme in the biosynthesis of all classes of flavonoids in plants. It catalyzes the stepwise condensation of three acetate residues from malonyl CoA with coumaroyl CoA [25].

Flavonoids are known to have important functions, for example as UV-light protectants [24, 53] and as phytoalexins in legumes [14, 15]. External stimuli such as stress, UV-light and attack by pathogens induce CHS expression in many plant species [12, 22, 33, 35, 50, 52].

The regulation of CHS expression by light has been studied most extensively in parsley cell cultures. UV-light is necessary for the transcription of the parsley CHS gene [7], but blue light and red light have modulating effects [6, 45]. UV-light regulation of CHS expression has also been reported for petunia [59], bean [50], *Antirrhinum*

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X16437.

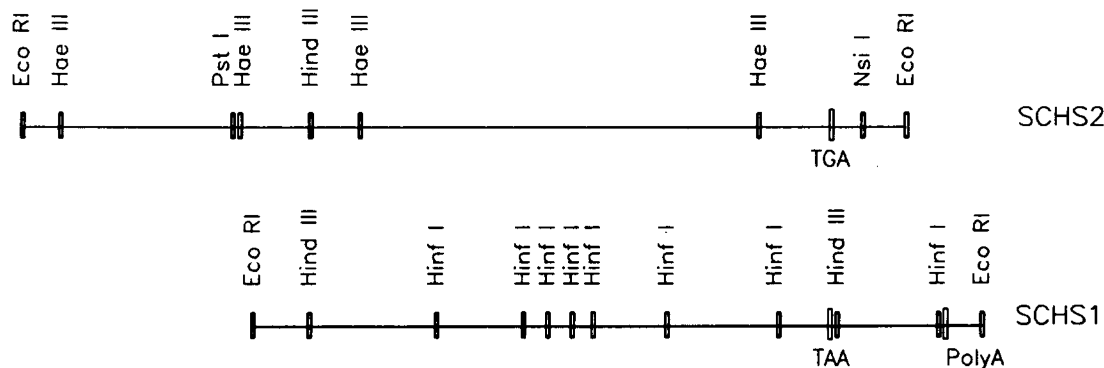


*Fig. 1.* Map of the mustard cDNA clones SCHS1 and SCHS2. Restriction sites within the clones and at the ends (generated by addition of *Eco* RI linkers) are indicated as the positions of stop codons (TGA, TAA). The four probes used for hybridization experiments were: 1. A 748 bp *Hin*d III fragment from the coding region of SCHS1; 2. A 139 bp *Hin*d III/*Hinf* I fragment from the 3′ noncoding region of SCHS1; 3. A 67 bp *Nsi* I/*Eco* RI fragment from the 3′ noncoding region of SCHS2; 4. A 300 bp *Eco* RI/*Pst* I fragment from the coding region of SCHS2. Cross-hybridization between the probes was not detectable even under low-stringency wash conditions.

from parsley [49]. Standard hybridization and washing conditions were used [27].

*DNA sequence analysis*

DNA sequencing was performed according to the dideoxy chain termination method using double-stranded DNA [33] cloned in pUC19. Overlapping subclones were obtained by *Exo* III/SI treatment [26].

*Primer extension experiments*

One oligonucleotide (5′ CCAACTTGGTTTTAACTAGAG 3′) was synthesized for primer extension experiments using a DNA synthesizer (Model 380B, Applied Biosystems). The oligonucleotide which is complementary to the coding strand starts 7 nucleotides upstream from the first ATG in the coding region. Primer extension experiments were performed as described [13].

## Results

*Isolation of genomic clones encoding the CHS*

$5 \times 10^5$ recombinants were screened with the $^{32}$P-labelled pLF15 cDNA probe coding for the parsley CHS [49]. After three rounds of plaque purification two clones were identified containing inserts of 10.6 (SA-CHS2) and 20.4 kbp (SA-CHS1). Both clones were characterized by restriction analysis (Fig. 2). Their restriction maps are similar but an additional *Hin*d III site is present in the coding region of SA-CHS2. No hybridization is detectable between SA-CHS2 and the 3′ noncoding region of the cDNA clone SCHS2 (data not shown). Therefore, SA-CHS2 is distinct from SA-CHS1 and from the two previously characterized CHS cDNAs from mustard. The clone SA-CHS1 was further characterized.

Double digestion with *Eco* RI and *Sal* I generates 5 fragments. The 7.9 and 1.7 kbp fragments hybridize with the pLF15 probe and were subcloned into pUC19. A *Hin*d III fragment of the mustard cDNA clone SCHS1 [18] (spanning positions 451 to 1198 relative to the translation start site) only hybridized with the 7.9 kbp *Eco* RI/*Sal* I fragment. Therefore, this fragment

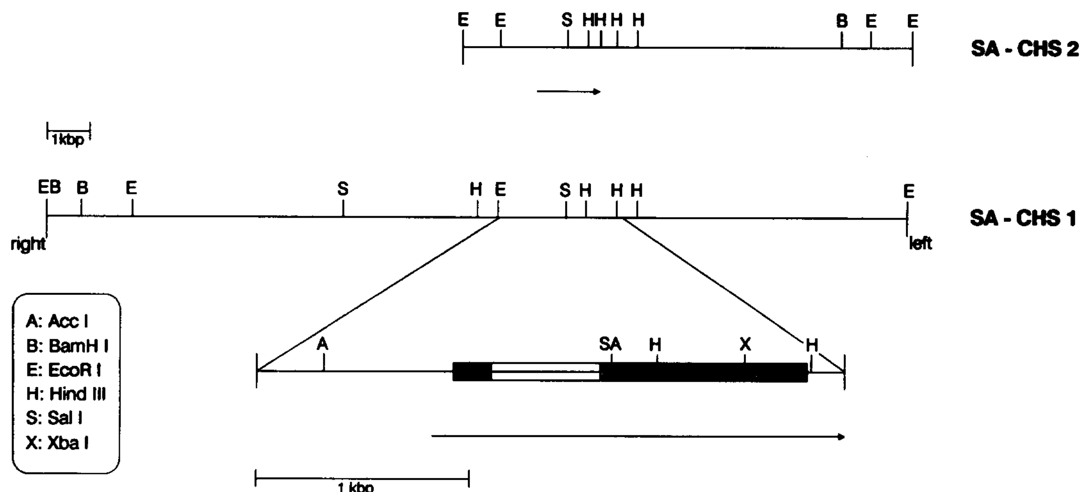


*Fig. 2.* Map of the mustard CHS clones SA-CHS1 and SA-CHS2. Restriction enzymes which were used to map the clones are indicated. The expanded region shows the 2.9 kbp sequenced part of SA-CHS1. Dark boxes indicate exons. The arrows indicate the location of the transcripts.

contains part of the coding region and the untranslated 3′ end.

The copy number of CHS genes was determined by Southern-blot analysis of mustard genomic DNA. The restriction enzymes *Eco* RI/*Sal* I and *Hind* III were used because they provided the most information from the restriction patterns of the genomic and cDNA clones. The data from the Southern analysis and the restriction patterns of the CHS clones indicate that four CHS genes are present in mustard (Fig. 3).

*Sequence of SA-CHS 1*

Part of the 7.9 kbp and all of the 1.7 kbp *Eco* RI/*Sal* I fragment were sequenced (Fig. 4).

The translation start site was identified by sequence comparison with the mustard cDNA clone SCHS3 [18] and CHS sequences from other species [19, 43]. SA-CHS1 encodes a polypeptide of 395 amino acids. The coding region is interrupted by a single intron of 523 bp between the first and second nucleotide of a Cys codon (codon 65). The position of this intron is conserved in all CHS genes analyzed so far [19, 27, 55]. The intron is bordered by 5′-GT and 3′-AG consensus sequences [3]. The G/C content (53%) is very similar to that of CHS from other dicot species [43].

Sequence comparisons of previously identified CHS cDNAs from mustard [18] and the genomic clone SA-CHS1 show that SA-CHS1 is the gene encoding the transcript from which one of these cDNAs (SCHS1) was synthesized. Furthermore, the coding region of the mustard gene is very similar to a recently published *Arabidopsis* CHS sequence [19] which also encodes a polypeptide of 395 amino acids. The percentage of homology between these two genes is 88% at the nucleotide level and 94.8% at the amino acid level.

208 bp of the untranslated 3′ end were sequenced. This region contains a putative polyadenylation signal [47], AATAAT, which is 135 bp downstream from the stop codon (TAA). It was not possible to define the poly(A) addition site exactly by comparison with the cDNA SCHS1 [18] because of the presence of 3 A residues in the genomic sequence at this site. The possible poly(A) addition sites are indicated in Fig. 4.

Primer extension experiments were performed to determine the transcription start site (Fig. 5) by using a 21mer oligonucleotide which was complementary to the coding strand starting from 7 bp upstream of the A in the start codon (ATG). The primer extension products were separated on se-

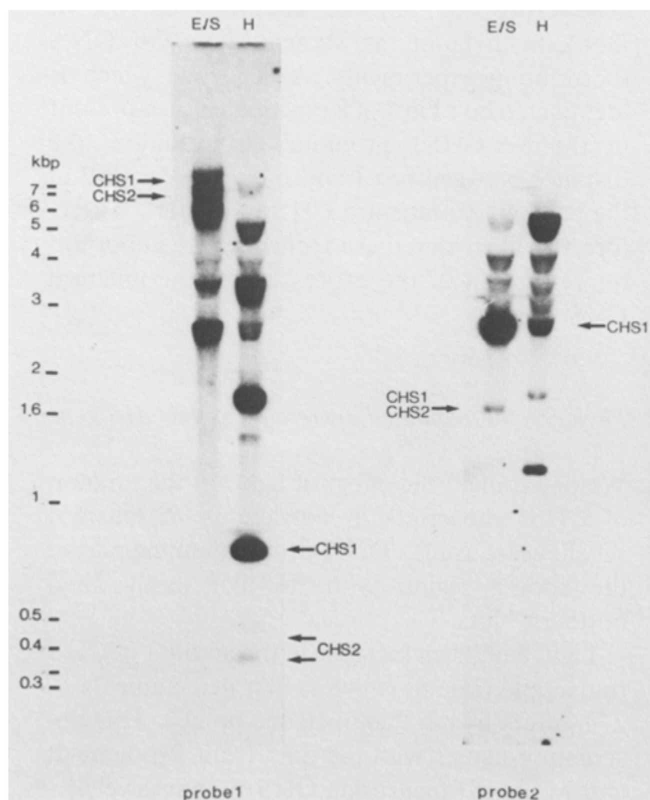


*Fig. 3.* Southern hybridization analysis. 20 μg of mustard genomic DNA was digested with the restriction enzymes indicated, separated on 0.7% agarose gels and blotted to Hybond N+ membranes. Filters were probed with radio-labelled CHS fragments (probe 1: 748 bp *Hin*d III fragment from SCHS1; probe 2: 300 bp *Eco* RI/*Pst* I fragment from SCHS2; see Fig. 1 for further details). Both probes represent nonoverlapping parts of the coding region. Bands which correspond to fragments from genomic clones are marked with arrows. Abbreviations: E: *Eco* RI; S: *Sal* I; H: *Hin*d III.

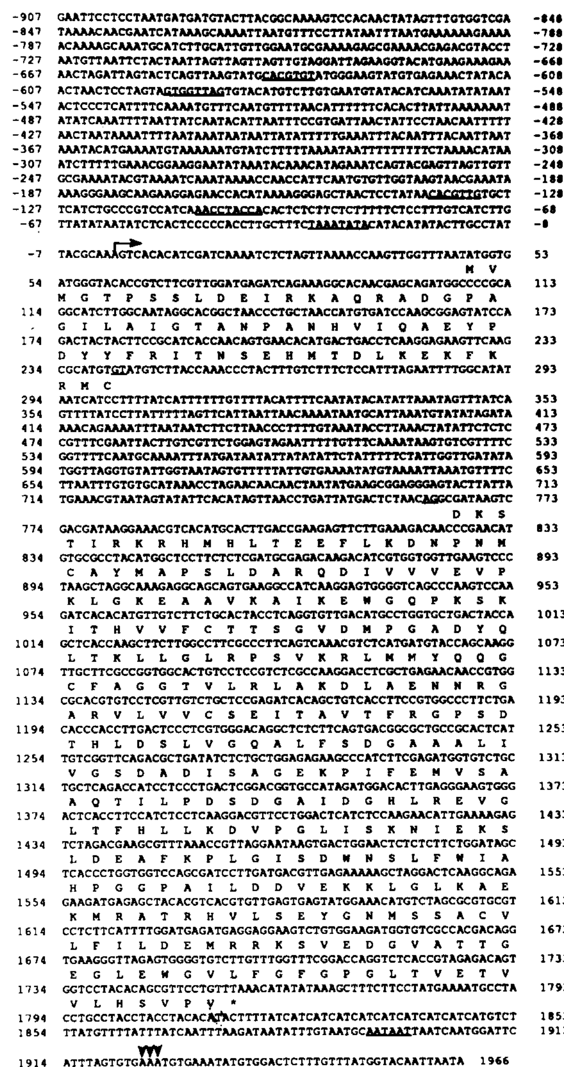

*Fig. 4.* Nucleotide and deduced amino acid sequence of the CHS1 gene from mustard. The transcription start (+1) is marked by a bent arrow. The putative TATA box is underlined as other areas which are discussed in the text. The possible poly(A) addition sites are marked by arrowheads.

quencing gels together with sequencing reactions using the same oligonucleotide and the 1.7 kbp *Eco* RI/*Sal* I fragment of SA-CHS1 in pUC19 as a template. The longest primer extension product corresponds to the third A residue (nucleotide +1, Fig. 4) in the sequence GCAAAGT. The presence of multiple bands in the primer extension experiments might be caused by premature termination of the reverse transcriptase.

A sequence motif (TAAATATA) which is similar to the TATA box [3] occurs at positions −34 to −27. In addition, another important sequence motif (GTGGTTAG) occurs at position −593 to −586. This motif is similar to the SV40 enhancer [54] and was also found in other CHS 5′ upstream sequences [19, 27, 55].

Another sequence motif (CACGTGT) is present in the mustard CHS promoter at position −640 (40 bp upstream of the motif which is

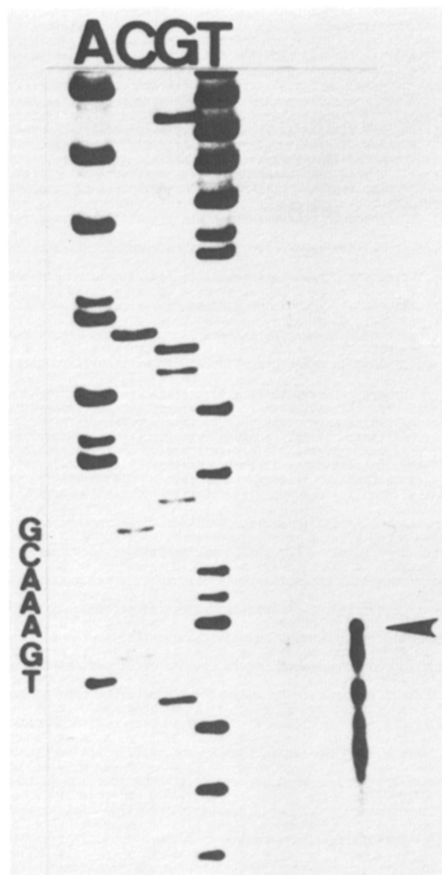


*Fig. 5.* Primer extension experiments to identify the 5′ end of the SA-CHS1 transcript. Primer extension products using 10 μg mustard RNA and 0.5 pmol primer (positions 21 to 41 in Fig. 4) were separated on 8% sequencing gels together with sequencing reactions using the same primer and the 1.7 kbp *Eco* RI/*Sal* I fragment of SA-CHS1. The arrowhead indicates the position of the longest primer extension product corresponding to the third A residue (position + 1) in the sequence GCAAAGT (see Fig. 4).

similar to the SV40 enhancer core sequence) and is repeated nearly identically at position − 138. This motif is present in all known CHS promoters and in most of the known *rbcS* promoters [53, 21, 39]. Furthermore, this sequence (G-box, box II) was found to specifically bind nuclear proteins from leaf tissue [21, 57]. In a paper recently published by Schulze-Lefert *et al.* [53] this motif and another one (box I; located 20 bp downstream from box II) were found to be essential for induction of the parsley CHS promoter in transient gene expression, and to be protected against DNA methylation as shown by *in vivo* DNA footprinting experiments. A sequence which is identical to box I in 7 of 8 positions is also present in the SA-CHS1 promoter. In addition, the distance between box I and box II is identical in the parsley and mustard CHS promoters. Therefore, it is likely that these sequences are important for regulation of the expression of the mustard CHS gene.

### *Phytochrome-regulated expression of the CHS gene*

We determined the effect of light on the amount of CHS transcripts in cotyledons of mustard seedlings by using a DNA-probe spanning part of the coding region with dot-blot assays and northern blots.

Light causes an increase in the amount of CHS transcripts (Fig. 6) which is first detectable 24 to 27 h after sowing ('competence point'). This observation agrees with the data from Brödenfeldt and Mohr [5] measuring CHS protein levels.

In dark-grown plants, a small but significant increase in CHS transcripts occurred between 36 and 42 h after sowing which is reflected in anthocyanin synthesis [44].

Treatment with continuous far-red light causes enhanced CHS transcript accumulation compared to continuous red light. This is a typical high irradiance response. The accumulation of CHS transcripts under continuous white light is less than with continuous far-red light. This observation (data not shown) makes it unlikely that UV/blue light photoreceptors are involved in CHS expression in mustard cotyledons. These effects were also described for anthocyanin accumulation [35, 58].

To test red/far-red light reversibility, an operational criterion for phytochrome action, mustard seedlings were irradiated from sowing on with continuous red light for 27 h and transferred to darkness for another 15 h or given a RG9 pulse and then transferred to darkness. A single 5 min RG9 light pulse after the red light treatment is sufficient to reduce the CHS-transcript level to

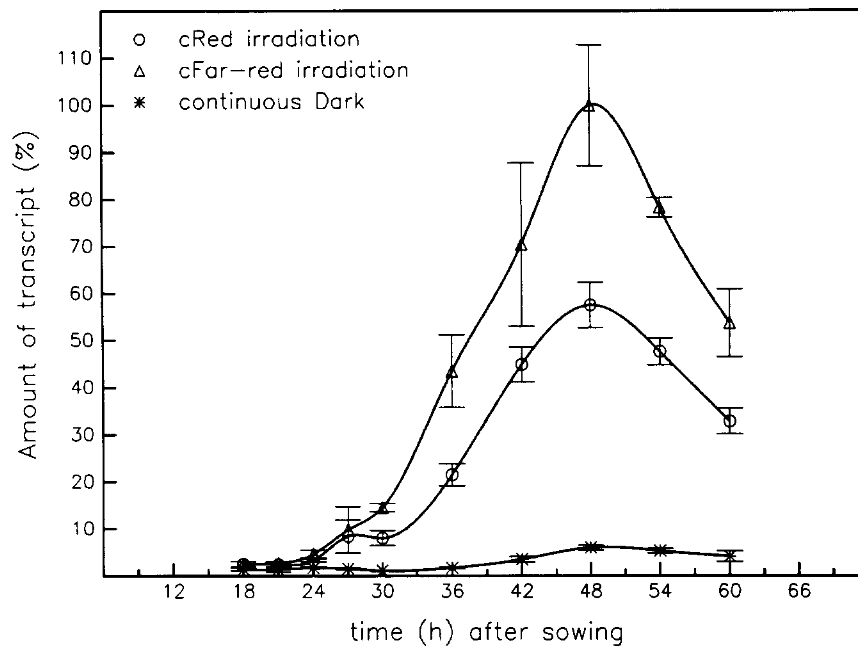


*Fig. 6.* Kinetics of CHS-mRNA accumulation in cotyledons. Plants were irradiated from sowing on with continuous red (cR) or far-red (cFR) light, and harvested at time points indicated for RNA isolation and dot-blot analysis.

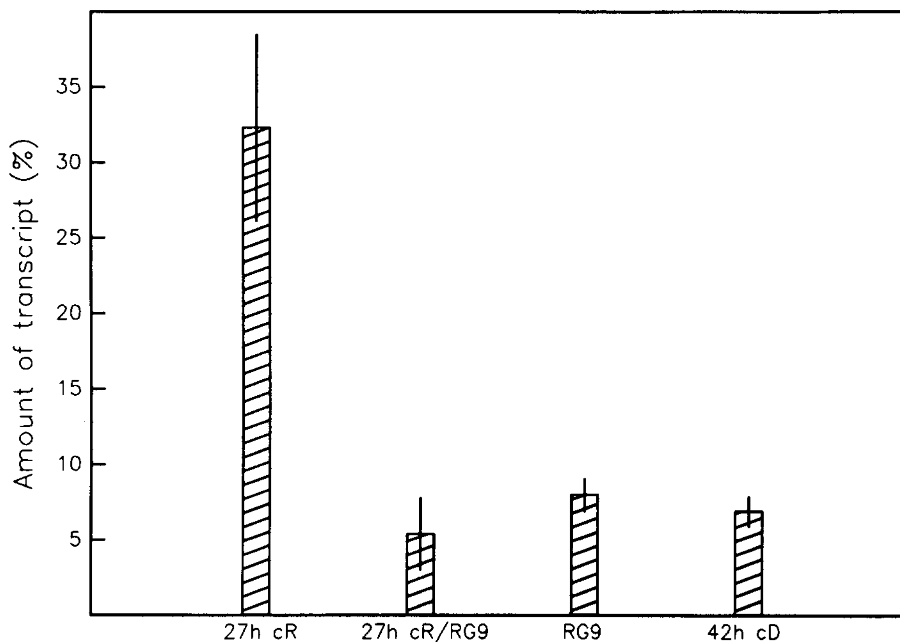


*Fig. 7.* Demonstration of phytochrome action. Plants were kept in continuous red light for 27 h from sowing on and either transferred to darkness directly (27h cR) or were given a 5 min RG9 pulse directly after the irradiation with red light (27h cR/RG9). Control etiolated plants received a RG9 pulse only 27 h after sowing (RG9) or were kept in darkness for 42 h (42h cD). All plants were harvested 42 h after sowing for RNA isolation and dot-blot analysis. Bars include highest and lowest values of three parallel measurements. The values are in percent of CHS transcripts accumulated after 42 h in cR light.

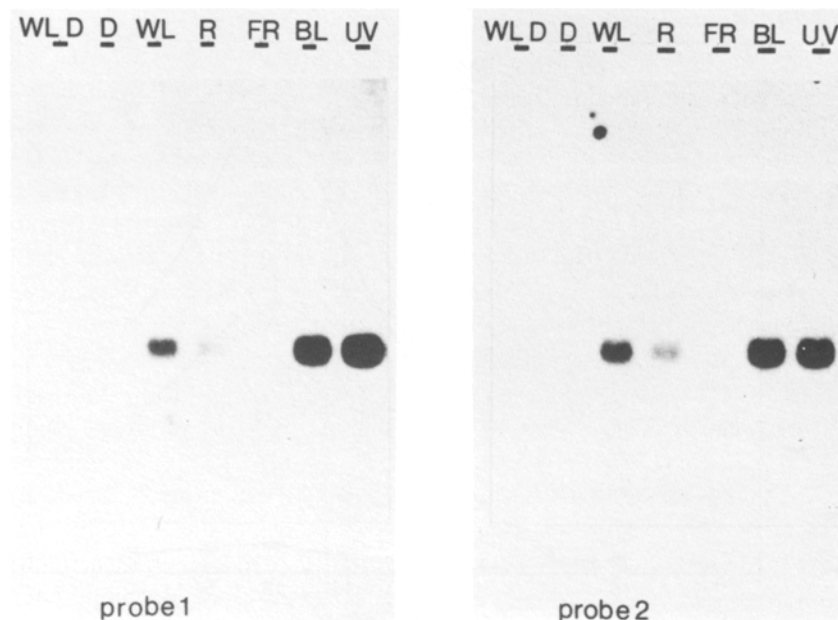


*Fig. 8.* Light regulation of CHS expression in primary leaves. Plants were grown for 23 d in a light/dark cycle (16 h white light/8 h darkness: WLD) and then transferred to darkness for 3 d (D). Afterwards, plants were treated with different lights for 16 h. 15 μg per lane of total RNA was separated on 1.2% agarose-formaldehyde gels transferred to nitrocellulose filters and hybridized with gene-specific probes: probe 1 was from SCHS1 and probe 2 from SCHS2. For further details see Fig. 1 and Materials and methods. Abbreviations: WLD: white-light/dark cycle for 23 d (samples taken at the end of light phase); D: plants kept in darkness for 3 d after the WLD cycle; WL: white light; R: red light; FR: far-red light; BL: blue light; UV: UV-light.

the dark control (Fig. 7). Identical regulation is seen by using a gene-specific probe for SA-CHS1 (data not shown).

Therefore, the regulation by red and far-red light of the accumulation of SA-CHS1 transcripts in mustard cotyledons can be interpreted as being exclusively by phytochrome action.

### *Blue and UV-light regulated expression of the CHS gene*

In order to determine if other photoreceptors can regulate CHS expression, we analyzed blue/UV-light regulation of CHS in primary leaves of mustard. Plants were grown for 23 d in a 16 h white light/8 h dark cycle, transferred to darkness for 3 d, and then given 16 h of different types of light. Northern blots using gene-specific SCHS1 and SCHS2 probes indicate that the CHS transcript level is very low after the 23 d white light/dark cycles and is undetectable after the 3 d dark period (Fig. 8). Blue and UV light caused maximal reinduction of CHS expression. Red light led to a much smaller increase in CHS transcript levels. There was no detectable effect of far-red light. These results indicate that blue and UV light regulate CHS expression in primary leaves.

## Discussion

Many different laboratories have studied the flavonoid pathway with genetic and biochemical approaches [8, 16, 23]. Most of the enzymes which are involved in the biosynthesis of the different classes of flavonoids (anthocyanins, flavones, flavonoles, and isoflavonoids) have been identified and partially purified. The first and key

enzyme in this pathway is the chalcone synthase. Thus, CHS genes have become an important tool for studying gene regulation in plants.

In order to understand the molecular mechanisms for CHS regulation, genes [19, 27, 31, 48, 50, 55, 60] and cDNAs [18, 43, 49, 50] have been isolated from different species including bean, barley, maize, *Petunia*, *Magnolia liliiflora*, *Ranunculus acer*, *Antirrhinum*, parsley, *Arabidopsis*, and mustard (this paper).

Several different groups have analyzed *cis*-acting elements in CHS promoters. One approach was to investigate different promoter constructs of the *Antirrhinum* CHS gene in tobacco cells [30] or in transient gene expression assays with parsley protoplasts [37]. Using this method, a minimal promoter size was defined which was sufficient for light regulation of the reporter gene. Sommer *et al.* [56] used transposon-induced deletions in the *Antirrhinum* CHS promoter to identify *cis*-acting elements which are necessary for CHS expression but seem not to be involved in light regulation. Using the transposon tagging method, it was possible to isolate a regulatory gene which is involved in CHS expression in maize endosperm [10, 46]. The expression in this tissue is not light-regulated.

The gene we have analyzed and described in this paper encodes a protein of 395 amino acids and is highly homologous to the *Arabidopsis* enzyme (88% on the nucleotide level and 94.8% on the amino acid level). It is interrupted by an intron of 523 bp at a position which is apparently conserved in all CHS genes. G-box (box II) like elements occur in the 5′ region of this gene. In addition, another sequence in the mustard CHS promoter is highly homologous to box I from the parsley CHS promoter. The distance between box I and box II is identical in both genes. The importance of these motifs in the induction of the parsley CHS gene in transient gene expression assays [53] strongly suggests that they are also important in regulating the mustard CHS gene.

A problem in analyzing light regulatory elements in CHS genes is that most species regulate CHS gene expression with several photoreceptors. For example, Koes *et al.* [32] demonstrated that the expression of the CHS-A gene from *Petunia hybrida* (V30) requires UV-light in seedlings but that the same gene is regulated by red light in floral tissue. Our results are similar in that red light regulates CHS1 expression in cotyledons, but blue and UV-light regulate CHS1 expression in primary leaves.

Although other photoreceptors beside phytochrome may influence CHS1 expression in cotyledons and phytochrome may modulate CHS1 expression in primary leaves, our experiments strongly suggest that there is a developmental switch in the hierarchy of photoreceptors which are involved in CHS1 regulation. Therefore the CHS1 gene is a good candidate to further characterize on a molecular level the photo-regulation and the interaction between different photoreceptors during plant development.


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## References


1. Beerhues L, Robenek H, Wiermann R: Chalcone synthases from spinach (*Spinacia oleracea* L.). II Immunofluorescence and immunogold localization. Planta 173: 544–553 (1988).
2. Beggs CJ, Kuhn K, Böcker R, Wellmann E: Phytochrome-induced flavonoid biosynthesis in mustard (*Sinapis alba* L.) cotyledons. Enzymic control and differential regulation of anthocyanin and quercetin formation. Planta 172: 121–126 (1987).

3. Breathnach R, Chambon P: Organization and expression of eucaryotic split genes coding for proteins. Ann Rev Biochem 50: 349–383 (1981).
4. Brödenfeldt R, Mohr H: Use of immunotitration to demonstrate phytochrome-mediated synthesis de novo of chalcone synthase and phenylalanine ammonia-lyase in mustard seedling cotyledons. Z Naturforsch 41c: 61–68 (1986).
5. Brödenfeldt R, Mohr H: Time courses for phytochrome-induced enzyme levels in phenylpropanoid metabolism (phenylalanine ammonia-lyase, naringenine-chalcone synthase) compared with time courses for phytochrome-mediated end-product accumulation (anthocyanin, quercitin). Planta 176: 383–390 (1988).
6. Bruns B, Hahlbrock K, Schäfer E: Fluence dependence of the ultraviolet-light-induced accumulation of chalcone synthase mRNA and effects of blue and far-red light in cultured parsley cells. Planta 169: 393–398 (1986).
7. Chappell J, Hahlbrock K: Transcription of plant defence genes in response to UV light or fungal elicitor. Nature 311: 76–78 (1984).
8. Chen SM, Coe Jr EH: Control of anthocyanin synthesis by the c-locus in maize. Biochem Genet 15: 333–346 (1977).
9. Chirgwin JM, Przybyla AE, Mac Donald RJ, Rutter WJ: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18: 5294–9299 (1979).
10. Cone KC, Burr FA, Burr B: Molecular analysis of the maize anthocyanin regulatory locus C1. Proc Natl Acad Sci USA 83: 9631–9635 (1986).
11. Cosio EG, Weissenböck G, McClure JM: Acifluorfen-induced isoflavonoids and enzymes of their biosynthesis in mature soybean leaves. Plant Physiol 78: 14–19 (1985).
12. Dangl JL, Hauffe KD, Lipphardt S, Hahlbrock K, Scheel D: Parsley protoplasts retain differential responsiveness to u.v. light and fungal elicitor. EMBO J 6: 2551–2556 (1987).
13. Dean C, Favreau M, Dunsmuir P, Bedbrock J: Confirmation of the relative expression levels of the *Petunia* (Mitchell *rbcS* genes. Nucl Acids Res 15: 4655–4668 (1987).
14. Dixon RA, Dey PM, Lamb CJ: Phytoalexins: Enzymology and molecular biology. Adv Enzymol 55: 1–136 (1983).
15. Dixon RA: The phytoalexin response: elicitation, signalling and control of host gene expression. Biol Rev 61: 239–291 (1986).
16. Dooner HK: Coordinate genetic regulation of flavonoid biosynthetic enzymes in maize. Mol Gen Genet 189: 136–141 (1983).
17. Douglas C, Hoffmann H, Schulz W, Hahlbrock K: Structure and elicitor or u.v.-light-stimulated expression of two 4-coumarate : CoA ligase genes in parsley. EMBO J 6: 1189–1195 (1987).
18. Ehmann B, Schäfer E: Nucleotide sequence encoding two different chalcone synthases expressed in cotyledons of SAN 9789 treated mustard (*Sinapis alba* L.). Plant Mol Biol 11: 869–870 (1988).
19. Feinbaum RL, Ausubel FM: Transcriptional regulation of the *Arabidopsis thaliana* chalcone synthase gene. Mol Cell Biol 8: 1985–1992 (1988).
20. Frischauf AM, Lehrach H, Poustka A, Murray N: Lambda replacement vectors carrying polylinker sequences. J Mol Biol 170: 827–842 (1983).
21. Giuliano G, Pichersky E, Malik VS, Timko MP, Scolnik PA, Cashmore AR: An evolutionarily conserved protein binding sequence upstream of a plant light-regulated gene. Proc Natl Acad Sci USA 85: 7089–7093 (1988).
22. Grab D, Loyal R, Ebel J: Elicitor-induced phytoalexin synthesis in soybean cells: Changes in the activity of chalcone synthase mRNA and the total population of translatable mRNA. Arch Biochem Biophys 243: 523–529 (1985).
23. Hahlbrock K, Grisebach H: Enzymatic controls in the biosynthesis of lignin and flavonoids. Ann Rev Plant Physiol 30: 105–130 (1979).
24. Hahlbrock K, Kreuzaler F, Ragg H, Fautz E, Kuhn DN: In: Jaenicke L (ed.). Biochemistry of differentiation and morphogenesis, pp 34–43. Springer, Berlin (1982).
25. Heller W, Hahlbrock K: Highly purified 'flavanone synthase' from parsley catalyzes the formation of naringenin chalcone. Arch Biochem Biophys 200: 617–619 (1980).
26. Henikoff S: Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene 28: 351–359 (1984).
27. Herrmann A, Schulz W, Hahlbrock K: Two alleles of the single-copy chalcone synthase gene in parsley differ by a transposon-like element. Mol Gen Genet 212: 93–98 (1988).
28. Hrazdina G, Marx GA, Hoch HC: Distribution of secondary plant metabolites and their biosynthetic enzymes in pea (*Pisum sativum* L.) leaves. Plant Physiol 70: 745–748 (1982).
29. Jahnen W, Hahlbrock K: Differential regulation and tissue-specific distribution of enzymes of phenyl-propanoid pathways in developing parsley seedlings. Planta 173: 453–458 (1988).
30. Kaulen H, Schell J, Kreuzaler F: Light-induced expression of the chimeric chalcone synthase-NPTII gene in tobacco cells. EMBO J 5: 1–8 (1986).
31. Koes RE, Spelt CE, Mol JNM, Gerats AGM: The chalcone synthase multigene family of *Petunia hybrida* (V30): Sequence homology, chromosomal localization and evolutionary aspects. Plant Mol Biol 10: 159–169 (1987).
32. Koes RE, Spelt CE, Mol JNM: The chalcone synthase multigene family of *Petunia hybrida* (V30): differential, light-regulated expression during flower development and UV light induction. Plant Mol Biol 12: 213–225 (1989).
33. Kraft R, Tardiff J, Krauter KS, Leinwand LA: Using mini-prep plasmid DNA for sequencing double stranded

templates with sequenase. Bio Techniques 6: 544–547 (1988).
34. Kreuzaler F, Ragg H, Fautz E, Kuhn DH, Hahlbrock K: UV-induction of chalcone synthase mRNA in cell suspension cultures of *Petroselinum hortense*. Proc Natl Acad Sci USA 80: 2591–2593 (1983).
35. Lange H, Shropshire Jr W, Mohr H: An analysis of phytochrome-mediated anthocyanin synthesis. Plant Physiol 47: 649–655 (1971).
36. Lawton MA, Lamb CJ: Transcriptional activation of plant defense genes by fungal elicitor, wounding, and infection. Mol Cell Biol 7: 335–341 (1987).
37. Lipphardt S, Brettschneider R, Kreuzaler F, Schell J, Dangl JL: UV-inducible transient expression in parsley protoplasts identifies cis-elements of a chimeric *Antirrhinum majus* chalcone synthase gene. EMBO J 7: 4027–4033 (1988).
38. Maniatis T, Fritsch EF, Sambrook J: Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982).
39. Manzara T, Gruissem W: Organization and expression of the genes encoding ribulose-1,5-bisphosphate carboxylase in higher plants. Photosynth Res 16: 117–130 (1988).
40. Mohr H: Untersuchungen zur phytochrominduzierten Photomorphogenese des Senfkeimlings (*Sinapis alba* L.). Z Pflanzenphysiol 54: 63–83 (1966).
41. Murashige T, Skoog F: A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15: 473–497 (1962).
42. Murray MG, Thompson WF: Rapid isolation of high molecular weight plant DNA. Nucl Acids Res 8: 4321–4325 (1980).
43. Niesbach-Klösgen U, Barzen E, Bernhardt J, Rohde W, Schwarz-Sommer Z, Reif HJ, Wienand U, Saedler H: Chalcone synthase genes in plants: A tool to study evolutionary relationships. J Mol Evol 26: 213–225 (1987).
44. Oelmüller R, Mohr H: Induction versus modulation in phytochrome-regulated biochemical processes. Planta 161: 165–171 (1984).
45. Ohl S, Hahlbrock K, Schäfer E: A stable blue-light-derived signal modulates ultraviolet-light-induced activation of the chalcone-synthase gene in cultured parsley cells. Planta 177: 228–236 (1989).
46. Paz-Ares J, Ghosal D, Wienand U, Peterson PA, Saedler H: The regulatory c1 locus of *Zea mays* encodes a protein with homology to myb proto-oncogene products and with structural similarities to transcriptional activators. EMBO J 6: 3553–3558 (1987).
47. Proudfoot NJ, Brownlee GG: 3′ non-coding region sequences in eukaryotic messenger RNA. Nature 263: 211–214 (1976).
48. Reif HJ, Niesbach U, Deumling B, Saedler H: Cloning and analysis of two genes for chalcone synthase from *Petunia hybrida*. Mol Gen Genet 199: 208–215 (1985).
49. Reimold U, Kröger M, Kreuzaler F, Hahlbrock K: Coding and 3′ non-coding nucleotide sequence of chalcone synthase mRNA and assignment of amino acid sequence of the enzyme. EMBO J 2: 1801–1805 (1983).
50. Ryder TB, Hedrick SA, Bell JN, Liang X, Clouse SD, Lamb CJ: Organization and differential activation of a gene family encoding the plant defense enzyme chalcone synthase in *Phaseolus vulgaris*. Mol Gen Genet 210: 219–233 (1987).
51. Schäfer E: Kunstlicht und Pflanzenzucht. In: Albrecht H (ed) Optische Strahlungsquellen. Lexika Verlag, Grafenau (1977).
52. Schmelzer E, Jahnen W, Hahlbrock K: In situ localization of light-induced chalcone synthase mRNA, chalcone synthase, and flavonoid end products in epidermal cells of parsley leaves. Proc Natl Acad Sci USA 85: 2989–2993 (1988).
53. Schulze-Lefert P, Dangl JL, Becker-Andre M, Hahlbrock K, Schulz W: Inducible in vivo DNA footprints define sequences necessary for UV light activation of the parsley chalcone synthase gene. EMBO J 8: 651–656 (1989).
54. Serfling E, Jasin M, Schaffner W: Enhancers and eukaryotic gene transcription. Tends Genet 1: 224–230 (1985).
55. Sommer H, Saedler H: Structure of the chalcone synthase gene of *Antirrhinum majus*. Mol Gen Genet 202: 429–434 (1986).
56. Sommer H, Bonas U, Saedler H: Transposon-induced alterations in the promoter region affect transcription of the chalcone synthase gene of *Antirrhinum majus*. Mol Gen Genet 211: 49–55 (1988).
57. Staiger D, Kaulen H, Schell J: A CACGTG motif of the *Antirrhinum majus* chalcone synthase promoter is recognized by an evolutionarily conserved nuclear protein. Proc Natl Acad Sci USA 86: 6930–6934 (1989).
58. Steinitz B, Drumm H, Mohr H: The appearance of competence for phytochrome-mediated anthocyanin synthesis in the cotyledons of *Sinapis alba* L. Planta 130: 23–31 (1976).
59. van Tunen AJ, Koes RE, Spelt CE, van der Krol AR, Stuitje AR, Mol JNM: Cloning of two chalcone flavanone isomerase genes from *Petunia hybrida*: coordinate, light-regulated and differential expression of flavonoid genes. EMBO J 7: 1257–1263 (1988).
60. Wenng A: PhD Thesis, Universität Freiburg, FRG (1988).
61. Wienand U, Weydemann U, Niesbach-Klösgen U, Peterson PA, Saedler H: Molecular cloning of the c2 locus of *Zea mays*, the gene coding for chalcone synthase. Mol Gen Genet 203: 202–207 (1986).