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Chalcone synthase and its functions in plant resistance

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Abstract Chalcone synthase (CHS, EC 2.3.1.74) is a key enzyme of the flavonoid/isoflavonoid biosynthesis pathway. Besides being part of the plant developmental program the *CHS* gene expression is induced in plants under stress conditions such as UV light, bacterial or fungal infection. CHS expression causes accumulation of flavonoid and isoflavonoid phytoalexins and is involved in the salicylic acid defense pathway. This review will discuss CHS and its function in plant resistance.

Keywords Chalcone synthase · Flavonoids · Plant resistance

Introduction

During their life cycle, plants respond actively to stress by producing phytoalexins and other stress metabolites. Such stress can result from injuries caused by the attack of insects and microbes or by mechanical wounding, and can induce many distinctive biochemical changes. These include the production of protective compounds either at the site of injury, or systemically in distant unwounded tissues (Kuhn 1988; Bowles 1990; Ryan 1990). In plants, phenylalanine is derived from the precursor chorismate and leads to the flavonoid, phenylpropanoid and stilbenoid biosynthetic pathways. All are interesting in connection with plant defense but in this review we will focus on the flavonoid biosynthesis pathway and its key enzyme chalcone synthase (CHS).

CHS is a member of the plant polyketide synthase superfamily, which also includes stilbene synthase (STS), acridone synthase, pyrone synthase, bibenzyl synthase, and *p*-coumaroyltriacetic acid synthase (Sanchez 2008). Chalcone synthases, the most well known representatives of this family, provide the starting materials for a diverse set of metabolites (flavonoids) which have different and important roles in flowering plants, such as providing floral pigments, antibiotics, UV protectants and insect repellents (Hahlbrock and Scheel 1989). Flavonoids also have benefits for human health, as they exhibit amongst others cancer chemopreventive (Jang et al. 1997), antimitotic (Edwards et al. 1990), estrogenic (Gehm et al. 1997) antimalarial (Li et al. 1995) antioxidant (Jang et al. 1997) and antiasthmatic (Zwaagstra et al. 1997) activities.

Flavonoids are synthesized via the phenylpropanoid and polyketide pathway, which starts with the

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condensation of one molecule of CoA-ester of cinnamic acid or derivatives such as coumaric or ferulic acid, and three molecules of malonyl-CoA, yielding a naringenin chalcone as major product. This reaction is carried out by the enzyme chalcone synthase (CHS). The chalcone is isomerised to a flavanone by the enzyme chalcone flavanone isomerase (CHI). From these central intermediates, the pathway diverges into several branches, each resulting in a different class of flavonoids. Flavanone 3-hydroxylase (F3H) catalyzes the stereospecific 3ß-hydroxylation of (2S)-flavanones to dihydroflavonols. For the biosynthesis of anthocyanins, dihydroflavonol reductase (DFR) catalyzes the reduction of dihydroflavonols to flavan-3,4-diols (leucoanthocyanins), which are converted to anthocyanidins by anthocyanidin synthase (ANS). The formation of glucosides is catalyzed by UDP glucose-flavonoid 3-*O*-glucosyl transferase (UFGT), which stabilizes the anthocyanidins by 3-*O*-glucosylation (Harborne and Grayer 1994; Bohm 1998). An overview of the flavonoid pathway is presented in Fig. 1. Flavonoids play an important role in plant defense, and CHS as the gatekeeper of flavonoid biosynthesis plays an important role in regulating the pathway. In fact *CHS* gene expression is influenced by many stress and environmental factors such as UV, wounding or pathogen attack (Dixon and Paiva 1995; Gläßgen et al. 1998; Ito et al. 1997).

In this review we will evaluate the present understanding about CHS and its regulation in plant resistance.

Structure and mechanism of chalcone synthase

The chalcone synthase (CHS) enzyme—known as a type III polyketide synthase enzyme (PKS) is structurally and mechanistically the simplest PKS (Schröder 1997; Sanchez 2008). These enzymes function as homodimeric iterative PKS (monomer size of 42–45 kDa) with two independent active sites that catalyze a series of decarboxylation, condensation, and cyclization reactions (Tropf et al. 1995). The three dimensional structure of alfalfa CHS2 was studied intensively by Ferrer et al. (1999). The study revealed that each alfalfa CHS2 monomer consists of two structural domains. In the upper domain, there are four amino acids (Cys164, Phe215, His303, and Asn336) are present at the active site were defined as the catalytic machinery of CHS. The lower domain of CHS has a large active site providing space for the tetraketide required for chalcone formation (i.e., naringenin and resveratrol) from one *p*-coumaroyl-CoA and three malonyl-CoA (Fig. 2) (Jez et al. 2001a, b). Production of chalcone requires the condensation of one molecule of *p*-coumaroyl-CoA and three malonyl-CoA molecules which is catalyzed by CHS. It starts with the transfer of a coumaroyl moiety from a *p*-coumaroyl-CoA starter molecule to an active site cysteine (Cys164) (Lanz et al. 1991). Next, a series of condensation reactions of three acetate units derived from three malonyl-CoA molecules, each proceeding through an acetyl-CoA carbanion derived from malonyl-CoA decarboxylation, extends the polyketide intermediate. Following generation of the thioester-linked tetraketide, a regiospecific intramolecular Claisen condensation forms a new ring system to yield chalcone. In plants, chalcone isomerase (CHI) will convert the chalcone to (*2S*)-5,7,4′-trihydroxyflavanone (naringenin); however, spontaneous ring closure in vitro results in mixed enantiomers of naringenin (Hahlbrock et al. 1970*;* Jez et al. 2000). In vivo chalcone can convert to narigenin without need of CHI. Four amino acids (Cys164, Phe215, His303, and Asn336) situated at the intersection of the CoA-binding tunnel and the active site cavity play an essential and distinct role during malonyl-CoA decarboxylation and chalcone formation. Cys164 plays role as the active-site nucleophile in polyketide formation and elucidate the importance of His303 and Asn336 in the malonyl-CoA decarboxylation reaction. Phe215 may help orient substrates at the active site during elongation of the polyketide intermediate. (Jez et al. 2000). The general reaction mechanism of CHS is presented in Fig. 2.

Several other cyclization reactions are possible besides the one yielding a chalcone. In addition to the starter molecule *p*-coumaroyl-CoA, in vitro alfalfa CHS accepts other CoA-linked thioesters as alternate starter molecules to generate corresponding chalcones, tetraketide lactone, and triketide lactone products (Fig. 3). The substrates can be feruloyl-CoA, hexanoyl-CoA, phenylacetyl-CoA, benzoyl-CoA, butyryl-CoA, isobutyryl-CoA and isovaleryl-CoA. With the starter substrates *p*-coumaroyl-CoA and malonyl-CoA, CHS catalyzes an intramolecular

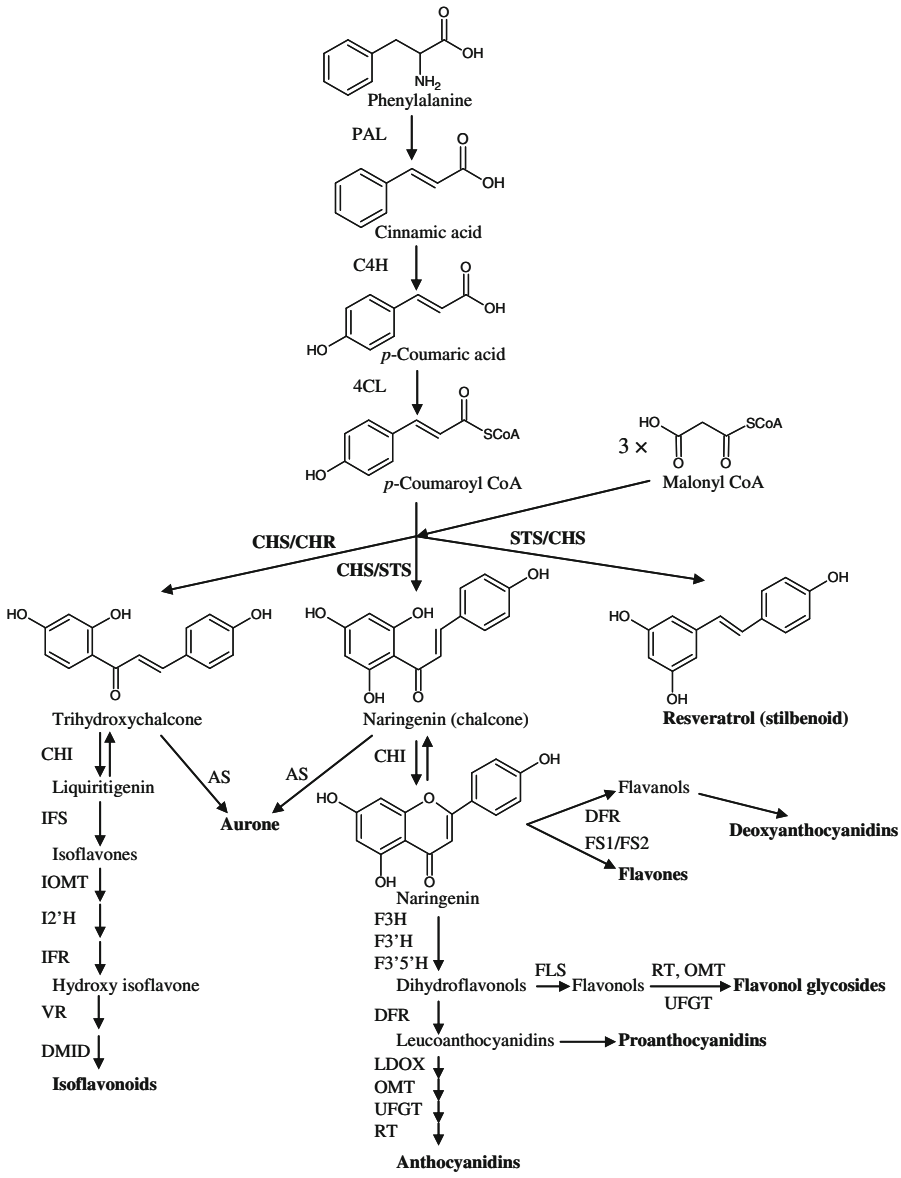

Fig. 1 Flavonoid biosynthetic pathway. *ANS* anthocyanidin synthase; *AS* aureusidin synthase; *C4H* cinnamate-4-hydroxylase; *CHR* chalcone reductase; *DFR* dihydroflavonol 4-reductase; *DMID* 7,2′-dihydroxy, 4′-methoxyisoflavanol dehydratase; *F3H* flavanone 3-hydroxylase; *F3′H* flavonoid 3′ hydroxylase; *F3′5′H* flavonoid 3′5′ hydroxylase; *FS1/FS2* flavone synthase; *I2′H* isoflavone 2′-hydroxylase; *IFR* isoflavone reductase; *IFS* isoflavone synthase; *IOMT* isoflavone *O*-methyltransferase; *LCR* leucoanthocyanidin reductase; *LDOX* leucoanthocyanidin dioxygenase; *OMT* *O*-methyltransferase; *PAL* phenylalanine ammonia-lyase; *RT* rhamnosyl transferase; *UFGT* UDP flavonoid glucosyl transferase; *VR* vestitone reductase; *STS* stilbene synthase; *FLS* flavanol synthase. (Winkel 1999; Yamaguchi et al. 1999; KEGG pathways)

Claisen condensation yielding the chalcone naringenin. Alfalfa CHS2 and parsley CHS (Hrazdina et al. 1976), accept feruloyl-CoA as a starter molecule and produce the tetraketide lactone (1b) and methylpyrone as the major products with the triketide lactone (1c) generated as a minor product. With hexanoyl-CoA, alfalfa CHS2 yields the tetraketide lactone (4b) as the major product, triketide lactone (4c) and methylpyrone are minor products (Jez et al. 2001a). Parsley CHS accepts butyryl-CoA and hexanoyl-CoA as substrates in vitro, which yield, respectively, the chalcone analogues, phlorobutyrophenone (5b) and phlorocaprophenone (4b) at pH 6.5 (Schuez et al. 1983). *Medicago sativa* CHS2 accepts phenylacetyl-CoA as a starter molecule yielding a phlorobenzyl ketone (2a), the chalcone-like product, accounts for less than 10% and others like tetraketide lactone (2b), triketide lactone (2c), and methylpyrone comprise the other products. The overall product distribution with phenylacetyl-CoA is similar to *Scutellaria baicalensis* CHS (Morita et al. 2000). With benzoyl-CoA as the starter molecule, alfalfa CHS2 generates

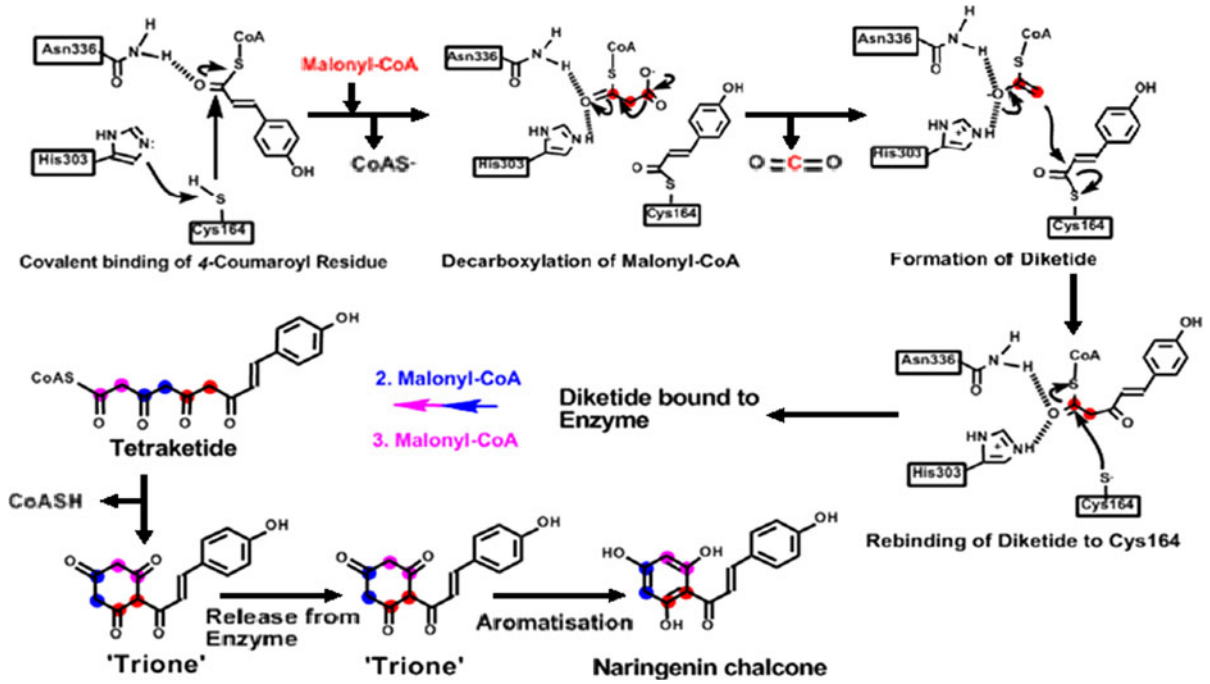

Fig. 2 Reaction catalyzed by chalcone synthase (CHS). In CHS, three amino acids play key roles in the catalytic functions of type III PKS: Cys164: active site, covalent binding site of starter residues and intermediates, His303 and Asn336: stabilization/activation of both starter (e.g. 4-coumarate) and extender units (malonyl-/acetyl-residues) (Ferrer et al. 1999; Bomati et al. 2005; modified by Schröder 2008)

phlorobenzophenone (3a) and methylpyrone as the major product, and tetraketide lactone (3b) and triketide lactone (3c) as minor products (Jez et al. 2001a). The recombinant hop CHS1 expressed in *E. coli* showed activity with isobutyryl-CoA and isovaleryl-CoA substrates, which produced as main products phloroisobutyrophenone (6b) and phloroisovalerophenone (7b) (Zuurbier et al. 1998; Novák et al. 2006).

The steady-state kinetic parameters of *Medicago sativa* CHS2 for *p*-coumaroyl-CoA, malonyl-CoA, feruloyl-CoA, hexanoyl-CoA, phenylacetyl-CoA and benzoyl-CoA have been determined, these are presented in Table 1 (Jez et al. 2001a; Novák et al. 2006).

Control of CHS activity

In plants, CHS is activated by a wide range of environmental and developmental stimuli. Theoretically, there are many ways to regulate CHS activity in vivo, from metabolic control to the control of initiation of transcription of the *CHS* gene (Martin 1993).

Metabolic control

There are many studies showing that CHS is inhibited noncompetitively by flavonoid pathway products like naringenin, chalcone naringenin and the other end products of CoA esters. For example, the parsley CHS is 50% inhibited by 100 µM naringenin and 10 µM CoA esters (Hinderer and Seitz 1985; Kreuzaler and Hahlbrock 1975), the flavonoids luteolin and apigenin are inhibitory to rye CHS in vitro (Peters et al. 1988), whereas in carrot, among the range of flavonoids tested, only naringenin and chalcone narigenin can inhibit CHS at 100 µM (Hinderer and Seitz 1985). It seems that flavonoids accumulate in the cytosol to a level that blocks CHS activity to avoid toxic levels for the plant (Whitehead and Dixon 1983), though there is no direct evidence that this inhibition happens in vivo.

Control of CHS turnover

In plants, CHS may always be present in the cells but is only activated under certain specific conditions. The statement "CHS may always be present in the cells but is only activated under certain specific conditions" means that CHS is activated at the protein level. However, it has been shown that UV light and biotic elicitors induce the flavonoid biosynthetic pathway at the transcriptional level and that CHS is not detectable before the onset of the various stress situations. Studies on parsley cell cultures showed that the induction of CHS activity by UV light was the result of de novo synthesis and active enzyme subsequently decayed with a half-life of 6 h, whereas inactive enzyme decayed more slowly with a half-life of 18 h (Schröder and Schäafer 1980). Inactive CHS could be detected by CHS antibodies and the size of the protein was not changed. In another study about accumulation of CHS during UV induction, Chappell and Hahlbrock (1984) concluded that the accumulation of flavonoid end products is presumably determined by activity of the rate-limiting step(s) in flavonoid biosynthesis and may not precisely reflect the dynamics of CHS activity in vivo.

Control of CHS through trans-genes

The activity of CHS can be controlled by antisense or sense genes. The studies on expression of antisense genes in *Petunia* (e.g. Van der Krol et al. 1988; Van der Meer et al. 1993), tobacco (Wang et al. 2006), *Gerbera hybrida* (Elomaa et al. 1996) and *Arabidopsis* (Le Gall et al. 2005) have shown that the presence of antisense *CHS* could inhibit the expression of the endogenous *CHS* in plants. In flowers of antisense *CHS* transgenic *Petunia,* the antisense construct was able to inhibit expression of the endogenous *CHS* genes to varying degrees, which is observed phenotypically as an inhibition of anthocyanin production to give completely acyanic or patterned flowers. In the cyanic sectors and flowers, transcripts of the endogenous *CHS* genes were under the detection limit, but the antisense transcripts were also barely detectable (Van der Krol et al. 1990b). The antisense effect most likely involves homologous pairing between the transcripts of endogenous *CHS* genes and transcripts of the introduced antisense *CHS* gene to form double stranded RNA that is very rapidly degraded, thus inhibiting *CHS* transcript accumulation and hence CHS activity.

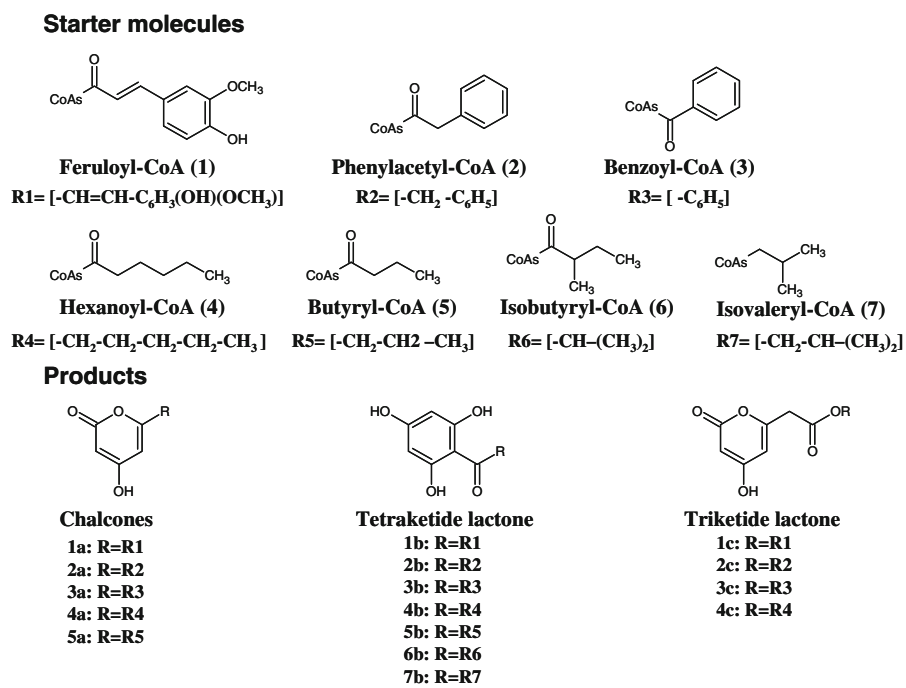

Fig. 3 Alternate starter molecules and their in vitro reaction products catalyzed by CHS

Table 1 Steady-state kinetic constants of *Medicago sativa* CHS2 with different starter substrates (Jez et al. 2001a; Novák et al. 2006)

	*k*cat (min^{-1})	*K*m (μM)
p-Coumaroyl-CoA	5.14 ± 0.30	6.1 ± 1.3
Malonyl-CoA	4.58 ± 0.24	4.7 ± 1.1
Feruloyl-CoA	1.04 ± 0.17	5.2 ± 0.9
Hexanoyl-CoA	2.52 ± 0.22	4.1 ± 1.2
Phenylacetyl-CoA	2.17 ± 0.35	5.1 ± 0.7
Benzoyl-CoA	1.73 ± 0.21	2.2 ± 0.2
Isobutyryl-CoA	–	14.9 ± 0.2
Isovaleryl-CoA	–	8.0 ± 0.2

Introducing a heterologous *CHS* gene in sense orientation can inhibit CHS activity in transgenic plants. This phenomenon is called co-suppression since it involves the reduction of transcriptional level of both endogenous and introduced genes in tissues where the endogenous gene is normally expressed (Napoli et al. 1990; Jorgensen 1995). This is known as gene silencing in which the transgene triggered not only its own silencing but also the endogenous chalcone synthase gene (Hammond et al. 2001). But on the other hand the introduced *CHS* gene may be expressed to high levels in tissue where the endogenous *CHS* genes are not expressed, such as in leaves of Petunia (Van der Krol et al. 1990a). Some studies have shown that co-suppression correlates with DNA methylation of the silenced sequences, presumably leading to a blockade at the transcriptional level or/and failure of transcript to accumulate in the cytoplasm resulting in a lack of enzyme activity (Ingelbrecht et al. 1994; Furner et al. 1998; Amedeo et al. 2000). Nowadays, the molecular mechanism of co-suppression of gene expression is thought to be related to the RNAi mechanism (Hannon 2002).

CHS localization and dynamics

The CHS protein in buckwheat (*Fagopyrum esculentum*) hypocotyls is located in the cytosol and associates with the cytoplasmic face of the rough endoplasmic reticulum (rER), but not with nuclei, plastids, mitochondria, Golgi, or tonoplasts (Hrazdina and Jensen 1992). Saslowsky and Winkel (2001) examined the subcellular location of CHS and CHI in *Arabidopsis* roots. High levels of both enzymes were found in the epidermal and cortex cells of the elongation zone and the root tip, consistent with the accumulation of flavonoid endproducts at these sites. Co-localization of CHS and CHI was observed at the endoplasmic reticulum and tonoplast in these cells.

However, there is evidence that flavonoids located in the nucleus may be synthesized in situ (Saslowsky and Winkel 2001). Several recent reports describe the accumulation of flavonoids in the nucleus in such diverse species as *Arabidopsis thaliana*, *Brassica napus*, *Flaveria chloraefolia*, *Picea abies, Tsuga canadensis,* and *Taxus baccata* (Buer and Muday 2004; Feucht et al. 2004; Grandmaison and Ibrahim 1996; Hutzler et al. 1998; Kuras et al. 1999; Peer et al. 2001). For the enzymes of the flavonoid pathway, several mechanisms may be involved. In the cytoplasm, flavonoid enzyme complexes are believed to assemble at the ER and in electron dense particles through the association of operationally-soluble enzymes such as CHS and CHI with the membrane-bound P450 hydroxylase, flavonoid 3′-hydroxylase (Saslowsky and Winkel 2001; Hrazdina and Wagner 1985). CHS possesses sequences resembling a classic nuclear localization signal (NLS). This signal is located on the surface, on the opposite side of the protein from the dimerization interface and could function to direct CHS, and perhaps associated enzymes into the nucleus. The localization of end products such as flavonol sulfate esters and flavan-3-ols to the nucleus suggests that additional flavonoid enzymes are also present in the nucleus (Grandmaison and Ibrahim 1996; Feucht et al. 2004).

There is an immuno gold-labeling study in grape berry showing that CHS was localized in rough endoplasmic reticulum (ER) and cytoplasm of the skin cells, while few gold particles were found on the cell wall. Besides, two novel sites of CHS were observed within cells of developing grape berry, one is in the plastids which remain unchanged throughout all stages of berry development. At the ripening stage of grape berry, CHS is present in the vacuole and in the vacuole membrane (tonoplast) (Tian et al. 2008). It is suggested that in grape berries, the synthesis of flavonoids in the ripening stage may occur in the vacuole.

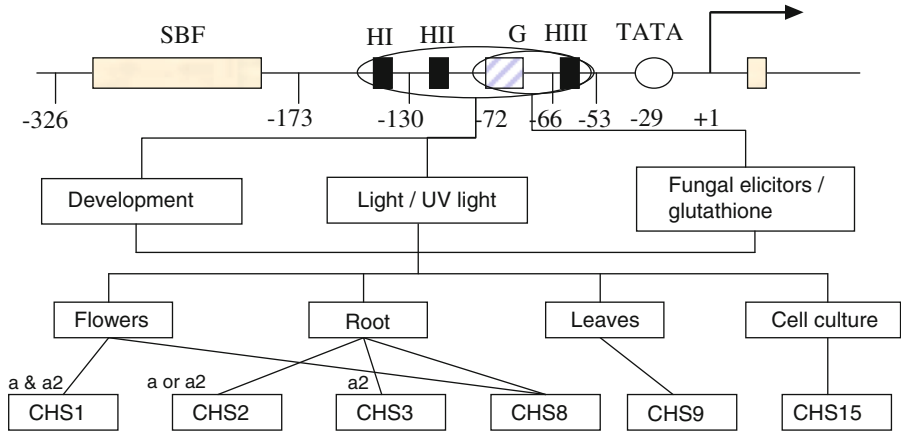

Fig. 4 Bean CHS15 promoter and regulators. *SBF* silencer binding factor, *H* H-Box (CCTACC), *G* G-Box (CACGTG), a/a2 regulation loci

Control of *CHS* gene expression

In *Arabidopsis*, parsley, and snapdragon only a single copy of the *CHS* gene has been found. In most angiosperms CHS has been shown to be encoded by a multigene family, such as in petunia (violet 30) (Koes et al. 1987), morning glories (Ipomoea) (Durbin et al. 2000), Gerbera (Helariutta et al. 1996), leguminous plants (Ryder et al. 1987; Wingender et al. 1989; Ito et al. 1997), and *Cannabis sativa* (Sanchez 2008).

Regulation of CHS gene expression

Many studies have shown that the *CHS* gene is constitutively expressed in flowers, but also its expression can be induced by light/UV light and in response to phytopathogens, elicitors or wounding in different parts of the plant, resulting in enhanced production of flavonoids (Koes et al. 1987; Ryder et al. 1984, 1987; Bell et al. 1986; Burbulis et al. 1996). *CHS* expression is also regulated by the circadian clock (Thain et al. 2002).

The level of *CHS* gene expression is reflected by the level of the *CHS* transcripts in plant cells. In order for transcription to take place, the RNA polymerase II must attach to specific DNA sequences in the *CHS* promoter in the vicinity of the TATA box and must be activated by specific DNA-binding proteins (transcription factors) binding to response elements further upstream in the promoter. The *CHS* promoter was studied extensively in *Phaseolus vulgaris, Antirrhinum, Arabidopsis,* and parsley (Dixon et al. 1994; Faktor 1997; Feinbaum et al. 1991; Lipphardt et al. 1988).

The *CHS* promoter contains the nucleotide sequence CACGTG regulatory motif known as G-box, which has been found to be important in the response to light/UV light (Kaulen et al. 1986; Staiger et al. 1989; Dixon et al. 1994; Schulze et al. 1989). Besides the G-box there are other domains in the *CHS* promoter involved in the light activation of *CHS* transcription. Those domains have been identified in the parsley *CHS* promoter as Box I, Box II, Box III, Box IV or three copies of H-box (CCTACC) in the *Phaseolus vulgaris CHS15* promoter. These boxes play a role as core promoter together with the G-box and all are required for light inducibility (Block et al. 1990; Lawton et al. 1990; Weisshaar et al. 1991).

The environmental and developmental control of CHS transcription has been investigated for the *CHS15* bean gene (Fig. 4) (Dixon et al. 1994; Harrison et al. 1991). The sequence elements required for transcriptional activation of the *CHS15* gene in response to fungal elicitors and glutathione are contained in a 130 bp region of the promoter (Choudhary et al. 1990; Dron et al. 1988; Harrison et al. 1991]). This region contains a G-box and H-box III. There is a silencer element located between positions −326 and −173 of the *CHS15* promoter (Dron et al. 1988). No *trans*-acting factors were found that could bind to *cis* elements in this region

but the region reduced expression of *CHS* (Harrison et al. 1991). An enhancer element was found in the *Antirrhinum CHS* promoter. It is located in the region between −564 and −647 and increased *CHS* gene expression in roots, stems, leaves, and seeds but not in petal tissue (Fritze et al. 1991).

The *Petunia CHSA* promoter was studied by van der Meer et al. (1990, 1993) to understand the role of the promoter in tissue-specific *CHS* expression. The studies showed that the promoter sequence between +1 and −67 confers flower specific *CHS* gene expression. Another study on the *Antirrhinum CHS* promoter has shown that the sequences between +1 and −39 allow *CHS* expression in root and stems, whereas sequences between −39 and −197 are required for expression in petals and seeds (Fritze et al. 1991).

The regulators of *CHS* in plants are controlled by some specific loci. In maize, there are four loci, *cl, r, vp,* and *clf*, involved in the regulation of *CHS* expression (Dooner 1983). Multiple regulatory loci for *CHS* expression have also been described for the petunia regulatory mutant Red Star. The phenotype of this mutant of red and white sectors in the flower petals is thought to depend on at least four regulatory genes, all of which regulate *CHS* expression in *trans* (Mol et al. 1983). In the *CHS* gene family of *Phaseolus vulgaris,* the regulation is via the *a* and *a2* loci though they regulate different *CHS* members in different ways. The *CHS* genes might have different combinations of *cis* elements that determine their response to the products of these regulatory loci. The expression of *CHSl* in flower tissue has an absolute requirement for the products of both the *a* and *a2* loci, whereas, in root tissue, the products of these loci are not required. It is possible that the *CHSl* gene interacts with one or more factors present in roots, which are absent in flowers, that can substitute for the products of the *a* and *a2* loci. *CHS3* expression in flower tissue is more complicated: it requires the product of the *a2* locus, but has a lower level of expression in *a* mutants compared with wild type. This suggests that *CHS3* interacts with both the *a2* and *a* locus products, but, unlike the *CHSl* gene, it may also interact with other products, allowing transcription at a low level in *a* mutants. *CHS2* is expressed in roots but not in petal tissue, suggesting that it may not be able to interact with the products of *a* and *a2* loci in petal tissue (Harker et al. 1990).

Transcription factors involved in of CHS gene expression

Trans-acting factors of bean *CHS15* that bind to two short sequences centered on the G-box and H-box also make major contributions to the in vivo transcription of the promoter (Arias et al. 1993; Yu et al. 1993). *Trans* activation required both a MYB-binding site and a G-box like element (Sablowski et al. 1994). MYB305, one of the MYB-like proteins that have been implicated in the transcriptional control of tissue-specific *CHS* gene expression, is also recognized by a *cis* element of the light-regulatory unit 1 (LRUI) of *CHS* in parsley (Feldbrügge et al. 1997). G-box/H-box binding factor 1(G/HBF-1), a basic leucine zipper (bZIP) protein, that binds to both the G-box and the adjacent H-box in the proximal region of the *CHS15* bean promoter, is rapidly phosphorylated in elicited soybean cells, this happen also to the *CHS15, CHS7,* and *CHS1* promoter (Dröge et al. 1997; Yoshida et al. 2008). Protein and mRNA levels of G/HBF-l do not change during the induction of *CHS* genes following pathogen attack (Yoshida et al. 2008) but *CHS* gene expression is strongly stimulated following phosphorylation responding to fungal elicitor treatment in vitro (Dröge et al. 1997).

CHS activity in plant resistance

In nature plants are exposed to a variety of biotic and abiotic stresses. Viruses, bacteria, fungi, nematodes and other pests attacking plants are biotic stresses, while light, temperature, wounding, drought, etc. are abiotic stresses. During stress conditions a plant is expressing a number of genes as part of its defense. Among these genes, CHS is quite commonly induced in different plant species under different forms of stress like UV, wounding, herbivory and microbial pathogens resulting in the production of compounds that have e.g. antimicrobial activity (phytoalexins), insecticidal activity, and antioxidant activity or quench UV light directly or indirectly. The current knowledge about regulation of CHS in plant pathogen resistance is presented in Table 2.

Table 2 Chalcone synthase expression in plant under stress conditions

No.	Host		Pathogen/stresses	Metabolites	References
1	*Petroselinum crispum*	Parsley	UV	Flavonoids	Schmelzer et al. (1988), Schulze et al. (1989)
2	*Phaseolus vulgaris* cells	French bean	*Colletotrichum lindemuthianu*		Ryder et al. (1984)
3	*Arabidopsis* cells		UV-B and UV-A/blue light		Christie and Jenkins (1996)
4	*Arabidopsis thaliana*		Low temperature	Anthocyanins	Leyva et al. (1995)
			UV-B, UV-A, and blue Light		Fuglevand et al. (1996), Hartmann et al. (1998), Wade et al. (2001)
			High-intensity lights	Anthocyanins	Feinbaum and Ausubel (1988)
			SA, ethylene, methyl jasmonate *Alternaria brassicicola*		Schenk et al. (2000)
			Pseudomonas syringae	Phenolic compounds	Soylu (2006)
5	*Petunia hybrida*		UV		Koes et al. (1989)
			Low temperature	Anthocyanin	Shvarts et al. (1997)
6	*Petroselinum hortense* cells		UV		Kreuzaler et al. (1983)
7	*Pinus sylvestris*	Scots pine	UV-B	Phenolic compounds, flavonoids, catechin	Schnitzler et al. (1996)
8	*Picea abies*	Norway spruce	*Ceratocystis polonica*		Nagy et al. (2004)
			Ophiostoma polonicum and wounding	Catechin	Brignolas et al. (1995)
9	*Secale cereale*		UV		Haussuehl et al. (1996)
10	*Hordeum vulgare*	Barley	*Blumeria graminis*		Christensen et al. (1998)
			Erysiphe graminis		
			UV		
11	*Medicago truncatula*	Alfalfa	*Glomus versiforme*	Isoflavonoid	Harrison and Dixon (1993)
	Medicago sativa				
12	*Antirrhinum majus*	Snapdragon	*Erwinia chrysanthemi*		Junghans et al. (1993)
			Rhizobium meliloti		
			$CuCl_2$		
			Wounding		
			Phoma medicaginis		
			Colletotrichum lindemuthianum		Dalkin et al. (1990)
			UV		Lipphardt et al. (1988)
					Staiger et al. (1989)
13	*Lycopersicon esculentum*	Tomato			
14	*Glycine max*	Soybean	*Pseudomonas syringae* pv *glycinea*		Dhawale et al. (1989)
			Phytophthora megasperma f. sp. *Glycinea*		
15	*Picea glauca*	White Spruce	Wounding, JA, MeJ		Richard et al. (2000)
16	*Daucus carota*	Carrot cell	UV, *Pythium aphanidermatum*	Anthocyanin	Gläßgen et al. (1998)

Table 2 continued

No.	Host		Pathogen/stresses	Metabolites	References
17	*Brassica rapa*	Turnip	UV	Anthocyanin	Zhou et al. (2007)
18	*Sorghum bicolor*	Sorghum mesocotyl, juvenile sorghum tissues	*Colletotrichum graminicola* *Helminthosporium maydis*	3-Deoxyanthocyanidins, apigeninidin luteolinidin	Lue et al. (1989), Nicholson et al. (1987)

Phytoalexins

Phytoalexins are antimicrobial metabolites produced by plants in response to microbial attack (or biotic and abiotic elicitors) (Dixon 1986). Phytoalexins come from many different metabolite classes such as flavonoids, stilbenoids, sesquiterpenoids, steroids and alkaloids. CHS can help the plant to produce more flavonoids, isoflavonoid-type phytoalexins and other related metabolites to protect it against stress. Accumulation of flavonoids and isoflavonoids in response to pathogen attack is seen in many plant species, and their importance as antimicrobial phytoalexins is well established (Matthews and Matthews 1989; Van Etten and Pueppke 1976). Flavonoid phytoalexins have been described in legumes, cereals, sorghum, rice, *Cephalocereus senilis, Beta vulgaris* (Hipskind et al. 1990; Johnson et al. 1976; Kodama et al. 1992; Pare et al. 1992). Some isoflavonoids were increased in *Lupin luteus* after infection with *Fusarium oxysporum* such as genistein, wighteone and luteon (Morkunas et al. 2005). The isoflavones, daidzein, genistein and glycitein, in soybean were strongly increased after infection by *Sclerotinia sclerotiorum* (Wegulo et al. 2005). Stilbenes are known as the phytoalexins in peanut (Ingham 1976) and grapes (Langcake and Pryce 1977a, b). There is also evidence that stilbene synthase (STS) has developed from CHS several times in the evolution (Tropf et al. 1994).

Phytoanticipins

Van Etten et al. (1995) defined phytoanticipins as low molecular weight, antimicrobial compounds that are constitutively expressed in plants without the need for infection with fungal pathogens or are produced after infection solely from preexisting constituents. The distinction between phytoalexins and phytoanticipins is not always clear as some compounds may be phytoalexins in one species and phytoanticipins in another species. Phytoanticipins also are classed into several chemical groups such as flavonoids, terpenoids, steroids, glucosinolates, and alkaloids.

The flavonoid epicatechin plays an important role as phytoanticipin in avocado fruits (Guetsky et al. 2005) and antimicrobial isoflavones desmodianones A, B and C have been isolated from *Desmodium canum* (Monache et al. 1996). Anthocyanins as products of the flavonoid metabolism are, for example responsible for the red to purple and blue colors of many fruits, vegetables, flowers, and cereal grains. In plants they serve as attractants for pollination and seed dispersal, give constitutive protection against the harmful effects of UV irradiation, and as phytoanticipins provide antiviral and antimicrobial activities in plants (Wrolstad 2000). Genotypes of *Ipomoea purpurea* with nonfunctional copies of chalcone synthase (*CHS*) received greater herbivore damage and twice the intensity of infection by the fungal pathogen *Rhizoctonia solani* than the wild type (Zufall and Rausher 2001).

Light protection

Phenolic compounds like flavonoids strongly absorb UV light and thus are able to protect plants from DNA damage caused by UV. Anthocyanins belong to a class of flavonoids that accumulate in leaves and stems as plant sunscreen in response to light intensity (Leyva et al. 1995). Expression of *CHS* genes is known to be regulated by light through a photoreceptor-mediated mechanism (Koes et al. 1989). In several cases, it was found that the photoregulated production of flavonoids is at least in part due to the transcriptional induction of *CHS* (Chappell and

Hahlbrock 1984; Feinbaum and Ausubel 1988; van Tunen et al. 1988; Taylor and Briggs 1990). Examination of *CHS* expression in parsley cell culture suggested that a UV-B light receptor, a blue light receptor and phytochrome may all play a role in light-induced *CHS* expression (Bruns et al. 1986; Ohl et al. 1989).

High intensity light and UV-A were found to regulate expression of chimeric chalcone synthase genes in transgenic *Arabidopsis thaliana* plants (Feinbaum et al. 1991). High-intensity light treatment of *A. thaliana* plants for 24 h caused a 50-fold increase in CHS enzyme activity and an accumulation of visibly detectable levels of anthocyanin pigments in the vegetative structures of these plants (Feinbaum and Ausubel 1988). The expression of *CHS* genes was increased with time during a 24 h exposure to UV-A on swollen hypocotyls of the red turnip 'Tsuda' and induced anthocyanin accumulation (Zhou et al. 2007). The flavonoids accumulate in epidermal cells of the leaves and it is specifically in these cells that *CHS* gene expression is induced by light stimuli (Schmelzer et al. 1988). However, in mustard the expression of two *CHS* genes is induced coordinately in seedlings grown in a dark environment for 36–42 h, though this induction is enhanced by supplying red or far red light (Ehmann et al. 1991).

Auxin and jasmonic acid signaling

In plant increase of CHS activity causes a high accumulation flavonoid level that inhibit polar auxin transport (Brown et al. 2001; Faulkner and Rubery 1992; Jacobs and Rubery 1988). Inhibitors of auxin transport could increase the resistance of tomato plants to *Fusarium oxysporum* (Davis 1954). Also other research showed that CHS is expressed in the nodule primordium and later primarily in uninfected cells of the nodule apex in *Rhizobium* infected legumes. This may explain the induction of nodule on infected legume roots, higher accumulation of flavonoids blocks auxin transport, causing a local accumulation of auxin, a growth hormone, which caused the induction of nodule growth and development (Estabrook and Sengupta 1991; Yang et al. 1992).

Jasmonic acid and its esters, such as methyl jasmonate (MeJA) are a group of plant hormones having a signaling role in insect and disease resistance (Xu et al. 1994). They could activate *CHS* in soybean and parsley cell cultures (Creelman et al. 1992) and *Picea glauca* (Richard et al. 2000). It is thought that volatile jasmonates are released from wounded tissue; thus elicitating plants to activate *CHS* which cause a production of phytoalexins in advance to resist an infection.

Conclusion

CHS is known as the key entry enzyme commited to the production of the polyketide phenylpropanoids in plants. It seems that all plants contain at least one *CHS* gene and often *CHS* gene families in plant with different expression patterns. In certain cases evolution into genes that encode enzymes with different substrate specificity, particularly for the starter molecule (e.g. aliphatic CoA ester instead of cinamic acid derivative) give different ring closure such as in stilbenes. The flavonoid pathway genes are highly diverted and have been found to be present from the earliest plants on land (the bryophytes, liverworts and hornworts) to the highly evolved flowering plants. Chalcones, flavonols and flavones were found in the earliest plants. Those flavonoids function as sunscreen protecting against UV radiation as plants began colonizing land and also play a regulation auxin transport (Markham, 1988; Shirley, 1996; Li et al. 1993; Brown et al. 2001). Later stage of plants such as the ferns and allies are known as oldest group of plants producing proanthocyanidins, procyanidin, prodelphinidin and flavanols. Anthocyanidin, a flavonoid, plays an important role in plant pigment action and is found in gymnosperms and angiosperms. These flavonoids serve diverse functions in different plant species, e.g. as pigments, phytoalexins, UV protectants, signal molecules in plant-microbe interactions, antioxidants, and pollinator attractants or feeding deterrents. In other words these unique plant compounds play a major role in the interaction of plants with their environment (De Bruyne et al. 1999; Kong et al. 2003: Marles et al. 2003; Yilmaz and Toledo 2004).

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