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Molecules of Interest

Flavones and flavone synthases

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Abstract

Within the secondary metabolite class of flavonoids which consist of more than 9000 known structures, flavones define one of the largest subgroups. Their natural distribution is demonstrated for almost all plant tissues. Various flavone aglyca and their *O*- or *C*-glycosides have been described in the literature. The diverse functions of flavones in plants as well as their various roles in the interaction with other organisms offer many potential applications, not only in plant breeding but also in ecology, agriculture and human nutrition and pharmacology. In this context, the antioxidative activity of flavones, their use in cancer prevention and treatment as well as the prevention of coronary heart disease should be emphasized. The therapeutic potential of flavones makes these compounds valuable targets for drug design, including recombinant DNA approaches. The biosynthesis of flavones in plants was found to be catalyzed by two completely different *flavone synthase* proteins (FNS), a unique feature within the flavonoids. The first, FNS I, a soluble dioxygenase, was only described for members of the Apiaceae family so far. The second, FNS II, a membrane bound cytochrome P450 enzyme, has been found in all other flavone accumulating tissues. This phenomenon is particularly of interest from the evolutionary point of view concerning the flavone biosynthesis and functions in plants. Recently, FNS I and FNS II genes have been cloned from a number of plant species. This now enables detailed biochemical and molecular characterizations and also the development of direct metabolic engineering strategies for modifications of flavone synthesis in plants to improve their nutritional and/or biopharmaceutical value.

Keywords: Flavonoids; Flavones; Flavone synthases; FNS

1. Introduction

Flavonoids represent a highly diverse class of secondary plant metabolites with about 9000 structures which have been identified up to now. These compounds are found in all vascular plants as well as in some mosses (Harborne and Baxter, 1999; Williams and Grayer, 2004). Even in the same species a number of different flavonoids may occur. It is already well established that flavonoids have a significant impact on various aspects of plant biology. They exhibit a wide range of functions in physiology, biochemistry, and ecology, for example in UV-protection, flower coloration, interspecies interaction, and plant defence. Moreover, for a long time flavonoid pattern are useful tools in phylogenetic studies. Other highly remarkably properties of certain flavonoids are their nutritional values and medicinal benefits to humans, represented among others by antioxidant or putative anticancer activities.

All flavonoids derive their 15-carbon skeletons from two basic metabolites, malonyl-CoA and *p*-coumaroyl-CoA. Basically, flavonoids are derivatives of 1,3-diphenylpropan-1-one (C_6–C_3–C_6). The crucial biosynthetic reaction is the condensation of three molecules malonyl-CoA with one molecule *p*-coumaroyl-CoA to a chalcone intermediate. Chalcones and dihydrochalcones are

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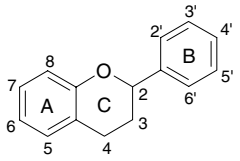

Fig. 1. Basic flavonoid structure.

classes of flavonoids that consist of two phenolic groups which are connected by an open three carbon bridge. Derived from the chalcone structure, a flavonoid-class containing three rings, the flavanones, can be formed. Here, the three-carbon bridge is part of an additional heterocyclic six-membered ring that involves one of the phenolic groups on the adjacent ring (for numbering convention to identify positions see Fig. 1). Based on these flavanones all other flavonoid-classes are generated, including isoflavones, flavanols, anthocyanidines, flavonols, and flavones (Fig. 2) (for review see Harborne and Baxter, 1999). This latter flavonoid-class is characterized by the presence of a double bond between C2 and C3 in the heterocycle of the flavan skeleton. The B-ring is attached to C2 and usually no substituent is present at C3. This exactly represents the difference to the flavonols where a hydroxyl group can be found at that C3 position. The term 'flavone' was used for the first time in 1895 by von Kostanecki and Tambor who were pioneers in the structural work of this particular class of flavonoids. Interestingly, higher plants evolved two completely independent enzyme systems to catalyze flavone synthesis using the same substrates. Both enzymes never occur side by side in the same organism: only in Apiaceae a soluble 2-oxoglutarate- and Fe^{2+}-dependent dioxygenase, flavone synthase I (FNS I), is present; on the other hand a NADPH- and molecular oxygen-dependent membrane bound cytochrome P-450 monooxygenase, flavone synthase II (FNS II), being more widespread among the plants, has been described (Heller and Forkmann, 1993).

Here, we first review the enzymatic mechanisms of both biosynthetic reactions catalyzed by the two flavone synthases, as well as the genetic and molecular aspects regarding their evolution. Moreover, the distribution, structural diversity and the various roles of flavones in the plants' physiology and ecology – with a focus on chemical communication with other organisms – and also their meaning for human health will be presented. At last, metabolic engineering strategies of flavone pathways will be discussed.

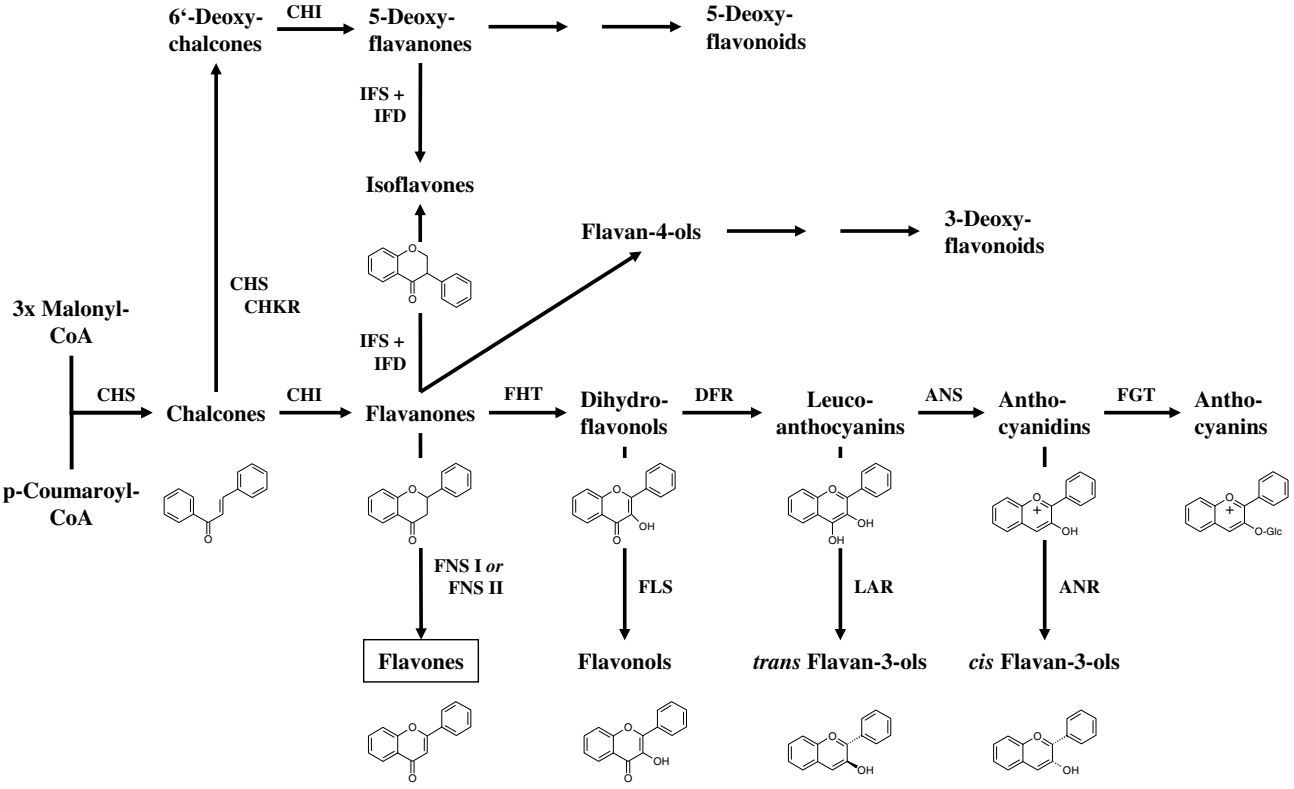

Fig. 2. Scheme of general flavonoid pathway. Enzymes are abbreviated as follows: CHS, chalcone synthase; CHKR, chalcone polyketide reductase; CHI, chalcone isomerase; FHT, flavanone 3-β-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; FGT, flavonoid glycosyltransferase; FNS, flavone synthase; FLS, flavonol synthase; LAR, leucoanthocyanidin reductase; ANR, anthocyanidin reductase; IFS, isoflavone synthase; IFD, isoflavone dehydratase.

2. Biosynthesis of flavones in plants

Flavones are synthesized at a branch point of the anthocyanidin/proanthocyanidin pathway from flavanones as the direct biosynthetical precursor (Fig. 2). At least two completely different proteins were found to be responsible for the particular enzymatic oxidative conversion, namely the introduction of a double bound between C2 and C3 by the abstraction of two hydrogen atoms (Fig. 3). Mainly, flavone formation in various tissues of a wide range of higher and lower plant species is catalyzed by the FNS II. All these enzymes belong to the superfamily of plant cytochrome P450 proteins, exactly to the subfamily CYP93B. FNS II activity was first demonstrated in snapdragon (*Antirrhinum majus*) flowers (Stotz and Forkmann, 1981) and osmotically stressed soybean (*Glycine max*) cell suspension cultures (Kochs and Grisebach, 1987). Detailed biochemical characterization of the native protein derived from crude plant extracts was further done with various plant species (for review see Forkmann and Heller, 1999). Moreover, in chemicogenetically defined genotypes of *Gerbera* hybrids (locus *Fns*) the correlation between genotype and enzyme activity for flavone formation could be demonstrated for the first time (Martens and Forkmann, 1998). Full length cDNAs of FNS II were recently cloned from *Gerbera* hybrids (CYP93B2) (Martens and Forkmann, 1999), *A. majus* (CYP93B3), *Torenia hybrida* (CYP93B4) (Akashi et al., 1999), *Perilla frutescens* (CYP93B6) (Kitada et al., 2001), *Callistephus chinensis* (CYP93B5) (Martens et al., unpublished), and *Gentiana triflora* (Acc. No. BAD91809) (Nakatsuka et al., 2005), respectively. The functionality of the heterologously expressed enzymes was proven in yeast cells. All recombinant proteins successfully converted flavanones to the corresponding flavones, apparently without any free intermediate thus indicating a direct conversion. Interestingly, another member of this cytochrome P450 subfamily, CYP93B1, has been identified as a flavanone 2-hydroxylase (F2H) in the legume *Glycyrrhiza echinata* (Akashi et al., 1998). Here the products, 2-hydroxyflavanones, were converted to flavones on acid treatment in vitro, suggesting the involvement of an additional enzyme, a dehydratase, to form a flavone in vitro (Akashi et al., 1998, 1999). Whether in *G. echinata* a FNS II is present besides the F2H remains to be elucidated. However, as the FNS II has been described for soybean (Kochs and Grisebach, 1987) it can be excluded that it represents the common biosynthetic pathway of flavones in Fabaceae.

The soluble FNS I enzyme has been described at first for parsley (*Petroselinum crispum*) cell suspension cultures and was classified as 2-oxoglutarate-dependent dioxygenase (Sutter et al., 1975; Britsch et al., 1981). The FNS I cDNA has been cloned from *P. crispum* and was functionally expressed in yeast by Martens et al. (2001). Up to now, FNS I appears to be confined to the plant family Apiaceae. This unique occurrence as well as its high sequence similarity to the flavanone 3-β-hydroxylase (FHT) laid the basis for evolutionary studies. The relationship between both proteins within the Apiaceae was recently investigated by molecular and phylogenetic analysis. Based on the results obtained, a gene duplication of FHT and subsequently a functional diversification was postulated. Most likely, this occurred early in the development of the Apiaceae subfamilies (Gebhardt et al., 2005).

3. Diversity and distribution of flavones

The flavones can be classified into several subgroups which are mainly indicated either by (i) hydroxylation, (ii) *O*-methylation, (iii) *C*-methylation, (iv) isoprenylation, or (v) methylenedioxy substitution. Besides the aglycon structures, *O*- and *C*-glycosides are well known. Flavones mostly occur as 7-*O*-glycosides, but substitution can be found at any other hydroxylated position. Chemically, flavonols are simply 3-hydroxyflavones. However, as flavones and flavonols are biosynthetically distinct flavonoid classes, only flavones will be discussed in the following. Because combinations of various modifications can occur, the number of different flavones is enormous. In 1999, Harborne and Baxter already listed more that 350 flavones and about 500 flavone glycosides indicating the high diversity of this flavonoid class (for an overview of the structures described see: Wollenweber, 1994; Harborne and Baxter, 1999; Williams and Grayer, 2004). Flavones can be found in all parts of the plants, above- and belowground, in vegetative and generative organs: stem, leaves, buds, bark, heartwood, thorns, roots, rhizomes, flowers, farina, fruit, seeds, and also in root and leaf exudates or resin.

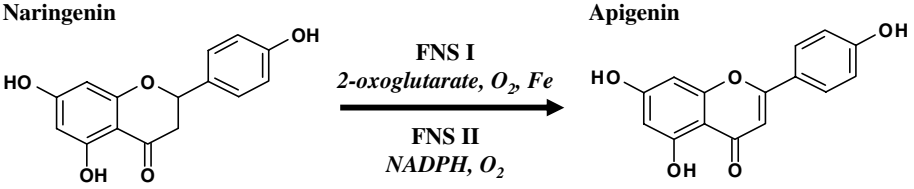

Fig. 3. Flavone formation catalyzed by flavone synthase I or II.

Flavones are present in all major land-plant lineages. The flavones-producing plant species belong to more than 70 different families within the plant kingdom (Harborne and Baxter, 1999). That means, at least some members of these plant families have conclusively been shown to synthesize flavones. Among them mainly plant families of higher plants are found. For example, the largest taxon with 64 different families to date is represented by the Angiosperms. But also within the Gymnosperms flavones have been described, at least in the Pinaceae and Cupressaceae. Beyond the Spermatophyta, flavones were isolated from species of lower plant taxa such as Pteridophyta (Dennstaedtiaceae, Equisetaceae, Isoetaceae, Lycopodiaceae) and Bryophyta (Hepaticae, Musci) (for reviews see Markham, 1988; Bohm, 1998). Whether or not algae are able to synthesize any flavonoids is still not clear (Bohm, 1998). Besides flavonols also two flavones, apigenin-(5,7,4′-trihydroxyflavone) *C*-glycosides, have been detected in fossils of *Quercus consimilis* found in the Succor Creek flora (Miocene, $25–17 \times 10^6$ years ago) of eastern Oregon (Niklas and Giannasi, 1978). These findings indicate that the ability of flavone synthesis is an evolutionary old feature of plants that was probably established when plants started to colonize land and identify flavones as an ancient class of phenylpropanoids.

In contrast to the nearly unique distribution of flavones within higher plants, these compounds seem to be absent in almost all of the about 3000 Brassicaceae species, although many other flavonoid-classes are present, especially flavonols. This conspicuous result coincides with the absence of genes encoding flavone synthases in the whole genome of the model plant *Arabidopsis thaliana*. Up to now, only few flavones have been found in Brassicaceae, all describing of 6-*C*-glycosides: one report identified two flavone glycosides in the leaves of *Barbarea vulgaris* (Senatore et al., 2000) and more recently, two studies showed their presence in *Isatis tinctoria* leaves (Cheng et al., 2005) and *Alliaria petiolata* seeds (Kumarasamy et al., 2004). In four other Brassicaceae species there are also evidences for the presence of flavones and flavone glycosides (Onyilagha et al., 2003). Another striking feature has been described for the plant family Apiaceae; here the FNS II is missing as well but its enzymatic activity has been substituted by the completely different enzymatic system, FNS I (Britsch, 1990; Martens et al., 2001). This peculiarity is unique within the flavonoid metabolism and underlines that the presence of flavone might be important for the plants.

4. Role of flavones in plants

It is well accepted that the flavonoids appeared when plants moved onto land. The high diversity of flavonoids that is established nowadays raises the question of the driving force behind this evolution. One criterion for selection might have been the ability of flavonoids, including flavones, to absorb UV radiation in the same range that is detrimental to nucleic acids; thus they can act as UV protection shield (Lowry et al., 1980). However, this view of a primary driving force has been challenged by Stafford (1991) who argued that originally the amount of those compounds would not have been sufficient. Besides this discussion, it is clear that in higher and lower plants flavone glycosides contribute to UV protection (Harborne and Williams, 2000). Another important feature of flavones that could have driven evolution is their allelochemical character. Flavones are involved in various interactions with other organisms, microbes as well as insects or other plants. For the host plant, these interactions can be both, beneficial or harmful. Whether or not flavones have been involved in the ancient symbiosis with mycorrhizal fungi is still uncertain. Very likely, the colonization of land and the evolution of land plants were strongly facilitated by intimate relations between plants and fungi where signal molecules are required (Brundrett, 2002; Kistner and Parniske, 2002). Thus, an involvement of flavonoids in general or flavones in particular is conceivable. Although in one report an inhibitory effect of such compounds on hyphal growth of the mycorrhiza fungus *Gigaspora* has been mentioned (Chabot et al., 1992), there are also evidences available supporting that hypothesis: Siqueira et al. (1991) showed that among other flavonoids the flavone, chrysin (5,7-dihydroxyflavone), can increase both mycorrhiza root colonization and root growth of *Trifolium repens*. Certain flavones have also been demonstrated to promote *Glomus* hyphal growth and spore germination (Tsai and Phillips, 1991). Interestingly, mycorrhizal associations with the roots of Brassicaceae are at best weak and facultative (Medve, 1983), and although arbuscular mycorrhizae have recently been reported from *Thlaspi*, it is doubtful if an effective symbiosis results (Regvar et al., 2003). Thus, it is tempting to speculate that one reason why roots of Brassicaceae are lacking mycorrhizae might be due to the absence of flavones' biosynthesis.

A defined role for flavones as signalling molecules has been realized in the symbiosis between legumes and nitrogen-fixing rhizobia. Among other flavonoids, flavones from the host roots are exuded and selectively recognized by the respective bacterial symbiont. Subsequently, the synthesis of bacterial nod-factor signals is initiated, which in turn are recognized by the plant host (Fisher and Long, 1992). The first flavone identified to participate in such a communication was luteolin (5,7,3′,4′-tetrahydroxyflavone) (Peters et al., 1986), but much more are described up to now (Broughton et al., 2000).

In plant insect interactions, flavones can be employed as co-pigments of delphinidin derivatives in

blue-flowered plants, the preferred flower colour of bees, thus contributing to the attraction of pollinators (Harborne and Williams, 2000). Again, in combination with a second compound that must be present, flavones (e.g., luteolin 7-*O*-(6″-*O*-malonyl)-β-D-glucoside together with *trans*-chlorogenic acid) are described to stimulate oviposition of certain insects on host plants (Feeny et al., 1988). Another aspect of flavones in plant insect interaction is their impact on feeding of herbivores. As reviewed by Simmonds (2003), flavones can affect insects in various ways, for instance they inhibit larvae feeding or act as feeding deterrent. Other flavones, such as 4″-hydroxy-maysin (5,7,3′,4′-tetrahydroxyflavone 2″-*O*-α-L-rhamnosyl-6-*C*-[6-deoxy-*xylo*-hexos-4-ulosyl]) can repress the development of corn earworm moth, *Heliothis zea*. Besides their effectiveness against insects, certain flavones also exhibit activities against a variety of different organisms: e.g., other plants (Basile et al., 2003; Beninger and Hall, 2005), nematodes (Soriano et al., 2004), molluscs (Lahlou, 2004), fungi (Wang et al., 1989; Weidenborner and Jha, 1997; Del Rio et al., 1998; Kong et al., 2004), oomycetes (Del Rio et al., 1998; Kong et al., 2004), and bacteria (Wang et al., 1989; Basile et al., 1999; Xu and Lee, 2001). These activities are often but not always and necessarily directed against attacking or phytopathogenic organisms. In such cases the flavones are frequently inducible and have to be classified as phytoalexins. Mostly these compounds are constitutively present in the particular plants. Unfortunately, no evident structure–function relationship has been identified that suggests why one flavone is involved in symbiotic communication, while others act as deterrents to herbivores or can be active as phytoalexins. However, a study by Picman et al. (1995) on the inhibition of mycelial growth of the fungus *Verticillium albo-atrum* as indicator of biological activities showed that totally unsubstituted flavone was more effective (in the range of 1–5 ppm) than substituted derivatives suggesting that at least the toxic effects might be due to hydrophobicity and their ability to interact with membrane structures.

5. Flavones in nutrition and health

Besides their important functions in the biochemistry, physiology and ecology of plants, flavones are important compounds for human nutrition and health. There is an increasing body of evidence for health-protecting functions of flavonoid compounds, such as antioxidative and antitumor effects in various cell lines, as well as antiinflammatory, antibacterial, antiviral, and antiatherosclerotic activities. Here, we will mainly focus on selected flavones regarding their anticancercogenic potential.

Flavones represent an abundant class of phytochemicals in our daily diet and are components found in edible vegetables, fruits, nuts, seeds and plant-derived beverages, such as juice and tea. Currently, they attract considerable scientific and therapeutic interest because of the assumed beneficial effect of flavone-containing food in the prevention of some human diseases. Epidemiology and animal studies suggested that a high dietary intake of flavonoids, including flavones, may be linked to a reduced risk of several cancers (e.g., lung and colon cancer), coronary heart disease, chronic inflammation, and osteoporosis (Middleton et al., 2000; Kromhout, 2001; Tabak et al., 2001; Ross and Kansum, 2002; Manach et al., 2003; Arts and Hollman, 2005). Flavones themselves have biochemical and pharmacological activities which are beneficial for human health, including antioxidant, anticancerogenic, anti-inflammatory, antiproliferative, antiangiogenic, and antiestrogenic effects, and ingestion produces no or very little toxicity (Havsteen, 2002). Although several authors tried to deduce structure–activity relationships for the cytotoxicity of flavonoids on cancer cell lines (e.g., Cao et al., 1997; Lopez-Lazaro et al., 2002 and references therein) such relationship was not obvious neither on basis of the subclasses nor with respect to groups or positions of substituents within a class (e.g., Kuntz et al., 1999). Because the biochemical activities depend on the individual flavone structures, each compound needs to be studied systematically to assess its individual biological potency. Additionally, comparative analyses with various flavonoids from different subgroups are necessary to examine anticancer activities of the most potent compounds.

The flavones baicalein (5,6,7-trihydroxyflavone) and its glucuronated derivative baicalin (baicalein 7-D-β-glucuronate) represent two dominant flavonoids from *Scutellaria baicalensis*, an important perennial herb in Chinese and Japanese traditional and clinical orientated medicine. *S. baicalensis* is used for the treatment of various types of cancer, hepatitis and T-cell leukemia but also diseases as fever, inflammation or several kinds of infections (Malikov and Yuldashev, 2002; Wozniak et al., 2004). Both compounds have strong antimutagenic and free radical scavenging properties (Wozniak et al., 2004). Baicalin is known to be converted in vivo to baicalein by the cleavage of the glycoside moiety. Recent studies provided evidence that both compounds not only exhibit activity against several cancers in vitro but also possess potent antiangiogenic potential both in vivo and in vitro. Characterization of the underlying mechanisms showed reduction of cell-associated matrix metalloproteinase-2 activity, inhibition of migration and proliferation, and in vitro capillary formation of vascular endothelial cells (Liu et al., 2003). The biological activity against prostata cancer was shown in vitro, in vivo and in patients with advanced stages of the disease applying the herbal medicine PC SPES, which is

known to contain baicalin as the most abundant active compound, as well as by the pure compound itself. Inhibition of proliferation was found also. In vitro, baicalin and baicalein caused an apparent accumulation of cells in G1, induced apoptosis, and decreased expression of the androgen receptor in LNCaP cells in a dose-dependent manner (Chen et al., 2001).

The more common plant-derived dietary flavone, apigenin, was found to be a potent inhibitor of cell proliferation and angiogenesis in human endothelial cells. The vascular endothelial growth factor expression was inhibited via degradation of hypoxia-inducible factor 1 alpha (Osada et al., 2004). Recently, it was reported that apigenin inhibited the growth of human cervical carcinoma cells (HeLa) and neuroblastoma cell lines, a pediatric tumor accounting for 15% of childhood cancer deaths. In apigenin-treated cervical cells, an induced p53 expression was detectable, which caused cell cycle arrest at G1 phase and apoptosis (Zheng et al., 2005). Cell number reduction and induction of apoptosis was found in a screening using apigenin together with other phytoestrogens in four prostate epithelial cells. It was found that apoptosis induction was caspase dependent. Besides the cleavage of several caspases, a loss of mitochondrial Bcl-2 expression, mitochondrial permeability, cytochrome *c* release and the cleavage of the inhibitor of apoptosis protein cIAP-2 was observed after apigenin treatment (Morrissey et al., 2005). Similar, for neuroblastoma the induction of caspase dependent, p53-mediated apoptosis upon treatment with the flavone has been found. Here, the presence of the C2–C3 double bound and the 4′-OH on the flavonoid structure correlated well with the growth-inhibitory effect of apigenin, indicating the therapeutic potential of flavones as antitumor agent (Torkin et al., 2005).

Surprisingly, flavone (2-phenyl-4H-1-benzopyran-4-one), the non-hydroxylated core structure of the flavone subgroup, was found to be a potent and selective inhibitor of proliferation, and an inducer of differentiation and apoptosis in colon cancer cells. Flavone proved to be a stronger apoptosis inducer than the clinically established antitumor agent camptothecin, a topoisomerase I inhibitor which is usually applied as a second-line pharmacotherapeutic in advanced colorectal cancers to promote apoptosis (Wenzel et al., 2000). Although the dietary importance of flavone is rather poor since its occurrence is only described in some cereal grains or in dill (Middleton and Kandaswami, 1993), the biochemical activities found so far are impressive. In HT-29 cells the mRNA levels of cell-cycle- and apoptosis-related genes including CDK-inhibitor p21, cyclooxygenase-2, nuclear transcription factor κB, and *bcl-*X_L was changed after treatment with flavone. Furthermore, flavone induces the activation of several caspases and a decrease of mitochondrial anti-apoptotic Bcl2 protein expression (Erhart et al., 2005). Cell arrest was found in a post-G1 phase before apoptosis occurs or differentiation is initiated in apoptosis-resistant cells. Moreover, flavone but not camptothecin, displayed a high selectivity for the induction of apoptosis and of growth inhibition only in the transformed but not in primary non-transformed colonocytes (Wenzel et al., 2000). The underlying mechanism of this selective activity is probably the enhanced uptake of monocarboxylates such as lactate into mitochondria most likely by an allosteric activation of monocarboxylate transporter-1 (Wenzel et al., 2005).

On the whole, these results suggest that non-mutagenic dietary flavones and in particular baicalein and its derivatives, apigenin, and flavone, have striking potential as agents for preventing several kinds of cancers.

6. Metabolic engineering strategies of flavone pathway in plants

Flavones become more and more commercially important for the floricultural, agricultural, food and pharmaceutical industry, respectively. In the latter case due to the dietary uptake of efficient antioxidants as described above. Consequently, genetic engineering can provide a valuable tool to expand the plant gene pool and thus promoting the generation of new commercial plant varieties or plant-derived products (e.g., food supplements, functional foods, herbal drugs). The biosynthesis of floral pigments, particularly anthocyanins, has been elucidated in great detail in several model flowers and is now being applied to other flowers providing means of targeting colour modification. Apart from flavone structures based on isoetin (5,7,2′,4′,5′-pentahydroxyflavone) which is described as yellow pigment in members of Chichoriae subfamily and in *Isoetes* spec. (Harborne, 1978) nearly all other flavones are acyanic visible only under UV-light. Generally, flavones and also flavonols contribute as common copigments to the colour appearance of cyanic pigments in plant tissues (see Section 4). Therefore, by metabolic engineering of this step the copigment level can be modified. However, as flavones and anthocyanins share common precursors, the levels of copigments and anthocyanin are generally negatively correlated. Practically, that means that a reduction on flavone level, e.g., by antisense suppression, will likely cause in increase of anthocyanin level due to precursor flow in only one direction. This assumption is further supported by a classical breeding experiment performed with chemicogenetically defined *Gerbera* line "Th 58" which comes out to be heterozygous for locus *Fns* (*fns*$^+$ *fns*) and only accumulates 4′-hydroxylated flavonoids. The self-crossing population segregated into three groups which could be classified by their pigment pattern and level (apigenin and pelargonidin), relative FNS II enzyme activity (high, medium,

x S

Th 58 [*fns*$^+$.]

147-120 [*fns*$^+$ *fns*$^+$]

147-125 [*fns*$^+$ *fns*]

147-120 [*fns fns*]

Pigment pattern	Pg, Ap	Pg, Ap	Pg
Pigment level	Pg : 0,05*; Ap : > 2,5 µg/ml	Pg : 0,3*; Ap < 2,5 µg/ml	Pg : 0,9*; n.d.
Enzyme activity[1]	> 50%	< 50%	n.d.
Genotype	*fns*$^+$ *fns*$^+$	*fns*$^+$ *fns*	*fns fns*

Fig. 4. Flavones as copigment. Self crossing of *Gerbera Hybrida* "Th 58" (Martens, 2000). Pg, pelargonidin; Ap, apigenin; *, absorption at 509 nm; n.d., not detectable; [1]relative conversion of ^{14}C-naringenin to ^{14}C-apigenin in standard FNS II assay (Martens and Forkmann, 1999).

no) and finally also by the genotype (*fns*$^+$ *fns*$^+$, *fns*$^+$ *fns*, *fns fns*) which was confirmed by generating F_2-populations from each group (Fig. 4) (Martens, 2000). However, down regulation of the FNS II gene in blue torenia (*Torenia Hybrida*) using antisense technique decreased the level of flavones as expected. Those of its precursor, the flavanones, are increased. Unexpectedly, the levels of anthocyanins were reduced and the resultant flower colour was pale blue (Ueyama et al., 2002). The strong copigment effect of flavones was recently documented in transgenic carnation (*Dianthus caryophyllus*). Florigene Moonshadow™, the first blue carnation, was analyzed regarding the flavonoid pattern. Besides delphinidin derivatives, the transgenic petals also contained an apigenin derivative that is proposed to have a strong copigment effect on the anthocyanin (Fukui et al., 2003).

In summary, both enzymes, FNS I and FNS II, enable the control of a biosynthetic step at an important junction of this pathway leading to various flavonoids classes, such as flavones, isoflavones, flavonols, flavanols and anthocyanins (Fig. 2). Thus, both genes might be useful in transgenic approaches to control the synthesis of these compounds by modulating the FNS activities. The direct regulation of flavone synthase activity or de novo synthesis of flavone formation in different plant tissues might be a useful tool to influence flower coloration and in addition a wide range of economic factors concerning plant performance including plants' disease resistance and nodulation capacity in legumes. Furthermore, the modification of flavonoid/flavone pattern in crops or pharmaceutical plants will lead to improved products for human nutrition and health as well.

References

Akashi, T., Aoki, T., Ayabe, S., 1998. Identification of a cytochrome P450 cDNA encoding (2S)-flavanone 2-hydroxylase of licorice (*Glycyrrhiza echinata* L.; Fabaceae) which represents licodione synthase and flavone synthase II. FEBS Letters 431, 287–290.

Akashi, T., Fukuchi-Mizutani, M., Aoki, T., Ueyama, Y., Yonekura-Sakakibara, K., Tanaka, Y., Kusumi, T., Ayabe, S., 1999. Molecular cloning and biochemical characterisation of novel cytochrome P450, flavone synthase II, that catalyzes direct conversion of flavanones to flavones. Plant and Cell Physiology 40, 1182–1186.

Arts, I.C., Hollman, P.C., 2005. Polyphenols and disease risk in epidemiologic studies. American Journal of Clinical Nutrition 81, 317–325.

Basile, A., Giordano, S., López-Sáez, J.A., Castaldo-Cobianchini, R., 1999. Antibacterial activity of pure flavonoids isolated from mosses. Phytochemistry 52, 1479–1482.

Basile, A., Sorbo, S., López-Sáez, J.A., Castaldo-Cobianchini, R., 2003. Effects of seven pure flavonoids from mosses on germination and growth of *Tortula muralis* HEDW. (Bryophyta) and *Raphanus sativus* L. (Magnoliophyta). Phytochemistry 62, 1145–1151.

Beninger, C.W., Hall, C., 2005. Allelopathic activity of luteolin 7-*O*-β-glucuronide isolated from *Chrysantemum morifolum* L. Biochemical Systematics and Ecology 33, 103–111.

Bohm, B.A., 1998. Introduction to Flavonoids. Harwood, Reading.

Britsch, L., 1990. Purification and characterization of flavone synthase I, a 2-oxoglutarate-dependent desaturase. Archives of Biochemistry and Biophysics 276, 348–354.

Britsch, L., Heller, W., Grisebach, H., 1981. Conversion of flananone to flavone, dihydroflavanol and flavonol with an enzyme system from cell cultures of parsley. Zeitschrift für Naturforschung 36c, 742–750.

Broughton, W.J., Jabbouri, S., Perret, X., 2000. Keys to symbiotic harmony. Journal of Bacteriology 182, 5641–5652.

Brundrett, M.C., 2002. Coevolution of roots and mycorrhizas of land plants. New Phytologist 154, 275–304.

Cao, G., Sofic, E., Prior, R.L., 1997. Antioxidant and prooxidant behaviour of flavonoids: structure–activity relationships. Free Radical Biology and Medicine 22, 749–760.

Chabot, S., Bel-Rhlid, R., Chênevert, R., Piché, Y., 1992. Hyphal growth promotion in vitro of the VA mycorrhizal fungus, *Gigaspora margarita* Becker & Hall, by the activity of structurally specific flavonoid compounds under CO_2-enriched conditions. New Phytologist 122, 461–467.

Chen, S., Ruan, Q., Bedner, E., Deptala, A., Wang, X., Hsieh, T.C., Traganos, F., Darzynkiewicz, Z., 2001. Effects of flavonoid baicalin and its metabolite baicalein on androgen receptor expression, cell cycle progression and apoptosis of prostate cancer cell lines. Cell Proliferation 34, 293–304.

Cheng, Y., Schneider, B., Oberthür, C., Graf, H., Adler, S., Hamburger, M., 2005. Flavone *C*-glycosides from *Isatis tinctoria* leaves. Heterocycles 65, 1655–1661.

Del Rio, J.A., Arcas, M.C., Benavente-García, O., Ortuño, A., 1998. Citrus polymethoxylated flavones can confer resistance against *Phytophthora citrophthora*, *Penicillium diditatum*, and *Geotrichum* species. Journal of Agricultural and Food Chemistry 46, 4423–4428.

Erhart, L.M., Lankat-Buttgereit, B., Schmidt, H., Wenzel, U., Daniel, H., Göke, R., 2005. Flavone initiates a hierarchical activation of the caspase–cascade in colon cancer cells. Apoptosis 10, 611–617.

Feeny, P., Sachdev, K., Rosenberry, L., Carter, M., 1988. Luteolin 7-*O*-(6″-*O*-malonyl)-β-D-glucoside and *trans*-chlorogenic acid: Oviposition stimulants for the black swallowtail butterfly. Phytochemistry 27, 3439–3448.

Fisher, R.F., Long, S.R., 1992. *Rhizobium*–plant signal exchange. Nature 357, 655–660.

Forkmann, G., Heller, W., 1999. Biosynthesis of flavonoids. In: Barton, D., Nakanishi, K., Meth-Cohn, O. (Eds.), Comprehensive Natural Products Chemistry. Elsevier Science Ltd., Oxford, pp. 713–748.

Fukui, Y., Tanaka, Y., Kusumi, T., Iwashita, T., Nomoto, K., 2003. A rationale for the shift in colour towards blue in transgenic carnation flowers expressing the flavonoid 3′,5′-hydroxylase gene. Phytochemistry 63, 15–23.

Gebhardt, Y., Witte, S., Forkmann, G., Lukacin, R., Matern, U., Martens, S., 2005. Molecular evolution of flavonoid dioxygenases in the family Apiaceae. Phytochemistry 66, 1273–1284.

Harborne, J.B., 1978. The rare flavone isoetin is a yellow flower pigment in Heywoodiella oligocephala and in other cichoriae. Phytochemistry 17, 915–917.

Harborne, J.B., Baxter, H., 1999Handbook of Natural Flavonoids, 2 vols. Wiley, Chichester.

Harborne, J.B., Williams, C.A., 2000. Advances in flavonoid research since 1992. Phytochemistry 55, 481–504.

Havsteen, B.H., 2002. The biochemistry and medical significance of the flavonoids. Pharmacology and Therapeutics 96, 67–202.

Heller, W., Forkmann, G., 1993. Biosynthesis of flavonoids. In: Harborne, J.B. (Ed.), The Flavonoids: Advances in Research since 1986. Chapman & Hall, London, pp. 499–535.

Kistner, C., Parniske, M., 2002. Evolution of signal transduction in intracellular symbiosis. TRENDS in Plant Science 7, 511–518.

Kitada, C., Gong, Z., Tanaka, Y., Yamazaki, M., Saito, K., 2001. Differential expression of two cytochrome P450s involved in the biosynthesis of flavones and anthocyanins in chemovarietal forms of *Perilla frutescens*. Plant and Cell Physiology 42, 1338–1344.

Kromhout, D., 2001. Diet and cardiovascular diseases. Journal of Nutrition, Health, and Aging 5, 144–149.

Kochs, G., Grisebach, H., 1987. Induction and characterization of a NADPH-dependent flavone synthase from cell cultures of soybean. Zeitschrift für Naturforschung 42c, 343–348.

Kong, C., Liang, W., Hu, F., Xu, X., Wang, P., Jiang, Y., Xing, B., 2004. Allelochemicals and their transformations in the *Ageratum conyzoides* intercropped citrus orchard soils. Plant and Soil 264, 149–157.

Kumarasamy, Y., Byres, M., Cox, P.J., Delazar, A., Jaspars, M., Nahar, L., Shoeb, M., Sarker, S.D., 2004. Isolation, structure elucidation, and biological activity of flavone 6-*C*-glycosides from *Alliaria petiolata*. Chemistry of Natural Compounds 40, 122–128.

Kuntz, S., Wenzel, U., Daniel, H., 1999. Comparative analysis of the effects of flavonoids on proliferation, cytotoxicity, and apoptosis in human colon cancer cell lines. European Journal of Nutrition 38, 133–142.

Lahlou, M., 2004. Study of the molluscicidal activity of some phenolic compounds: structure–activity relationship. Pharmaceutical Biology 42, 258–261.

Liu, J.-J., Huang, T.S., Cheng, W.-F., Lu, F.-J., 2003. Baicalein and Baicalin are potent inhibitors of angiogenesis: inhibition of endothelial cell proliferation, migration and differentiation. International Journal of Cancer 106, 559–565.

Lopez-Lazaro, M., Galvez, M., Martin-Cordero, C., Ayuso, M., 2002. Cytotoxicity of flavonoids on cancer cell lines: structure–activity relationship. In: Atta-ur-Rahman (Ed.), Studies in Natural Products Chemistry. Elsevier, Amsterdam, the Netherlands, pp. 891–932.

Lowry, B., Lee, D., Henabt, C., 1980. The origin of land plants: a new look at an old problem. Taxon 29, 183–197.

Malikov, V.M., Yuldashev, M.P., 2002. Phenolic compounds of plants of the *Scutellaria* L. genus. Distribution, structure, and properties. Chemistry of Natural Compounds 38, 473–519.

Manach, C., Mazur, A., Scalbert, A., 2003. Polyphenols and prevention of cardiovascular diseases. Current Opinion in Lipidology 16, 77–84.

Markham, K.R., 1988. Distribution of flavonoids in the lower plants and its evolutionary significance. In: Harborne, J.B. (Ed.), The Flavonoids: Advances in Research since 1980. Chapman & Hall, London, pp. 427–468.

Martens, S., 2000. Genetic, biochemical, and molecular biological investigations of flavone synthesis in Gerbera Hybrids. Ph.D. thesis, TUM Freising-Weihenstephan.

Martens, S., Forkmann, G., 1998. Genetic control of flavone synthase II activity in flowers of *Gerbera hybrids*. Phytochemistry 49, 1953–1958.

Martens, S., Forkmann, G., 1999. Cloning and expression of flavone synthase II from *Gerbera* hybrids. The Plant Journal 20, 611–618.

Martens, S., Forkmann, G., Matern, U., Lukačin, R., 2001. Cloning of parsley flavone synthase I. Phytochemistry 58, 43–46.

Medve, R.J., 1983. The mycorrhizal status of the Cruciferae. American Midland Naturalist 109, 406–408.

Middleton, E., Kandaswami Jr., C., 1993. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: Harborne, J.B. (Ed.), The Flavonoids: Advances in Research Since 1986. Chapman & Hall, London, pp. 619–652.

Middleton, E., Kandaswami Jr., C., Theoharides, T.C., 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological Reviews 52, 673–751.

Morrissey, C., O'Neill, A., Spengler, B., Christoffel, V., Fitzpatrick, J.M., Watson, R.W., 2005. Apigenin drives the production of reactive oxygen species and initiates a mitochondrial mediated cell death pathway in prostate epithelial cells. Prostate 63, 131–142.

Nakatsuka, T., Nishihara, M., Mishiba, K., Yamamura, S., 2005. Temporal expression of flavonoid biosynthesis-related genes regulates flower pigmentation in gentian plants. Plant Science 168, 1309–1318.

Niklas, K.J., Giannasi, D.E., 1978. Angiosperm Paleo-biochemistry of Succor Creek flora (Miocene) Oregon, USA. American Journal of Botany 65, 943–952.

Onyilagha, J., Bala, A., Hallett, R., Gruber, M., Soroka, J., Westcott, N., 2003. Leaf flavonoids of the cruciferous species, *Camelina sativa*, *Crambe* spp., *Thlaspi arvense* and several other genera of the family Brassicaceae. Biochemical Systematics and Ecology 31, 1309–1322.

Osada, M., Imaoka, S., Funae, Y., 2004. Apigenin suppresses the expression of VEGF, an important factor for angiogenesis, in endothelial cells via degradation of HIF-1alpha protein. FEBS Letters 575, 59–63.

Peters, N.K., Frost, J.W., Long, S.R., 1986. A plant flavone, luteolin, induces expression of *Rhizobium meliloti* nodulation genes. Science 233, 977–980.

Picman, A.K., Schneider, E.F., Picman, J., 1995. Effect of flavonoids on mycelial growth of *Verticillium albo-atrum*. Biochemical Systematics and Ecology 23, 683–693.

Regvar, M., Vogel, K., Irgel, N., Wraber, T., Hildebrandt, U., Wilde, P., Bothe, H., 2003. Colonization of pennycresses (*Thlaspi* spp.) of the Brassicaceae by arbuscular mycorrhizal fungi. Journal of Plant Physiology 160, 615–626.

Ross, J.A., Kansum, C.M., 2002. Dietary flavonoids: bioavailability, metabolic effects, and safety. Annual Review of Nutrition 22, 19–34.

Senatore, F., D'Agostina, M., Dini, I., 2000. Flavonoid glycosides in *Barbarea vulgaris* L. (Brassicaceae). Journal of Agricultural and Food Chemistry 48, 2659–2662.

Simmonds, M.S.J., 2003. Flavonoid–insect interactions: recent advances in our knowledge. Phytochemistry 64, 21–30.

Siqueira, J.O., Safir, G.R., Nair, M.G., 1991. Stimulation of vesicular–arbuscular mycorrhiza formation and growth of white clover by flavonoid compounds. New Phytologist 118, 87–93.

Soriano, I.R., Asenstorfer, R.E., Schmidt, O., Riley, I.T., 2004. Inducible flavone in oats (*Avena sativa*) is a novel defense against plant–parasitic nematodes. Phytopathology 94, 1207–1214.

Stafford, H.A., 1991. Flavonoid evolution: an enzymatic approach. Plant Physiology 96, 680–685.

Stotz, G., Forkmann, G., 1981. Oxidation of flavanones to flavones with flower extracts of *Antirrhinum majus* (snapdragon). Zeitschrift für Naturforschung 36c, 737–741.

Sutter, A., Poulton, J., Grisebach, H., 1975. Oxidation of flavanone to flavone with cell-free extracts from young parsley leaves. Archives of Biochemistry and Biophysics 170, 547–556.

Ueyama, Y., Suzuki, K., Fukuchi-Mizutani, M., Fukui, Y., Miyazaki, K., Ohkawa, H., Kusumi, T., Tanaka, Y., 2002. Molecular and biochemical characterization of *Torenia* flavonoid 3′-hydroxylase and flavone synthase II and modification of flower colour by modulating the expression of these genes. Plant Science 163, 253–263.

Tabak, C., Arts, I.C., Smit, H.A., Heedrik, D., Kromhout, D., 2001. Chronic obstructive pulmonary disease and intake of catechins, flavonols, and flavones: the MORGAN Study. American Journal Respiratory and Critical Care Medicine 164, 61–64.

Torkin, R., Lavoie, J.-F., Kaplan, D.R., Yeger, H., 2005. Induction of caspase-dependent, p53-mediated apoptosis by apigenin in human neuroblastoma. Molecular Cancer Therapy 4, 1–11.

Tsai, S.M., Phillips, D.A., 1991. Flavonoids released naturally from alfalfa promote development of symbiotic *Glomus* spores in vitro. Applied and Environmental Microbiology 57, 1485–1488.

von Kostanecki, S., Tambor, J., 1895. Ueber die Constitution des Fisetins. Berichte der Deutschen Chemischen Gesellschaft 28, 2302–2309.

Wang, Y., Hamburger, M., Gueho, J., Hostettmann, K., 1989. Antimicrobial flavonoids from *Psiadia trinervia* and their methylated and acetylated derivatives. Phytochemistry 28, 2323–2327.

Weidenborner, M., Jha, H.C., 1997. Antifungal spectrum of flavone and flavanone tested against 34 different fungi. Mycological Research 101, 733–736.

Wenzel, U., Kuntz, S., Brendel, M.D., Daniel, H., 2000. Dietary flavone is a potent apoptosis inducer in human colon carcinoma cells. Cancer Research 60, 3823–3831.

Wenzel, U., Schoberl, K., Lohner, K., Daniel, H., 2005. Activation of mitochondrial lactate uptake by flavone induces apoptosis in human colon cancer cells. Journal of Cellular Physiology 202, 379–390.

Williams, C.A., Grayer, R.J., 2004. Anthocyanins and other flavonoids. Natural Products Repopts 21, 539–573.

Wollenweber, E., 1994. Flavones and flavonols. In: Harborne, J.B. (Ed.), The Flavonoids – Advances in Research Since 1986. Chapman & Hall, London, pp. 259–335.

Wozniak, D., Lamer-Zarawska, E., Matkowski, A., 2004. Antimutagenic and antiradical properties of flavones from the roots of *Scutellaria baicalensis* Georgi. Nahrung/Food 48, 9–12.

Xu, H.-X., Lee, S.F., 2001. Activity of plant flavonoids against antibiotic-resistant bacteria. Phytotherapy Research 15, 39–43.

Zheng, P.W., Chiang, L.C., Lin, C.C., 2005. Apigenin induced apoptosis through p53-dependent pathway in human cervical carcinom cells. Life Science 76, 1367–1379.