Pergamon

PII: S0031-9422(98)00175-7

Phytochemistry, Vol. 49, No. 2, pp. 285–293, 1998

Printed in Great Britain
0031–9422/98/$–see front matter

REVIEW ARTICLE NUMBER 133

TEOSINTE TO MAIZE—SOME ASPECTS OF MISSING BIOCHEMICAL AND PHYSIOLOGICAL DATA CONCERNING REGULATION OF FLAVONOID PATHWAYS

IN HONOUR OF PROFESSOR G. H. NEIL TOWERS 75TH BIRTHDAY

HELEN A. STAFFORD

Biology Department, Reed College, Portland, Oregon 97202, U.S.A.

(*Received* 9 *October* 1997; 16 *January* 1998)

Key Word Index—flavonoid pathway; biochemistry, physiology; molecular genetics; structural genes; regulatory genes; maize; teosinte; evolutionary relationships.

Abstract—Flavonoid biochemical pathways, the end-products and their functions, molecular genetics, and the structural and regulatory genes in maize and teosinte are reviewed. Inadequate biochemical identifications and terminology problems are emphasized. Tissue-specific expressions of flavonoids in four tissues or organs of maize: anthers, pericarp, aleurone, and vegetative parts are discussed. Genes of the female inflorescence, considered critical in the evolutionary conversion of the teosinte vegetative body and floral structures into that of maize, have been studied. However, only a few genes of the C1 regulatory family controlling anthocyanin formation have been identified. Major biochemical and physiological data are still needed to understand this fascinating evolutionary sequence.

INTRODUCTION

Cultivated maize (*Zea mays* ssp *mays*) probably evolved from the annual teosinte sub-species, *Zea parviglumis*, about 8000 years ago [1]. In addition to the published genetic and developmental information about both groups, a more complete study of the phenolic compounds involved, including flavonoids, is crucial to understanding how this may have occurred. In this review, an emphasis upon the biochemical and physiological data about flavonoids in both maize and teosinte is given in order to help understand the molecular genetics involved.

FLAVONOID END-PRODUCTS, BIOSYNTHETIC PATHWAYS & MISSING LINKS IN MAIZE

The biosynthesis of the initial, basic C_{15} flavonoid molecule involves both the acetate pathway leading to malonyl-CoA and the phenylpropanoid route to 4-hydroxycinnamic acid, the two substrates of the first enzyme at the C_{15} level (Fig. 1) [2–4]. Malonyl-CoA from the acetate pathway provides the six carbons of the A-ring, whereas the nine carbons of the C- and B-rings come from 4-coumaroyl-CoA (CoA derivative of 4-hydroxycinnamic acid. The first enzyme at the C_{15} level, chalcone synthase (CHS), combines 3 malonyl-CoA molecules with one 4-coumaroyl-CoA (3 CO^2 molecules are released from the malonyl-CoA) (Fig. 2). The major flavonoids identified somewhat adequately in maize, mainly by paper and thin-layer chromatography, are the flavonols and 3-hydroxy-forms of anthocyanidins [5–7]. The flavan-3-ols and proanthocyanidins, 3-hydroxy type, might also be expected to be present, but have not been adequately identified. Furthermore, no isoflavonoids have been found in maize, and possible 3-deoxy products, flavan-

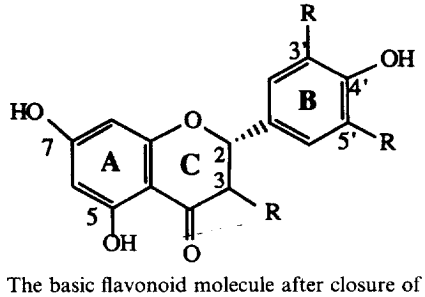

Fig. 1. The basic flavonoid molecule after closure of the C-ring: the A ring is derived from malonyl-CoA, the A and B-rings from 4-coumaroyl-CoA [2–4].

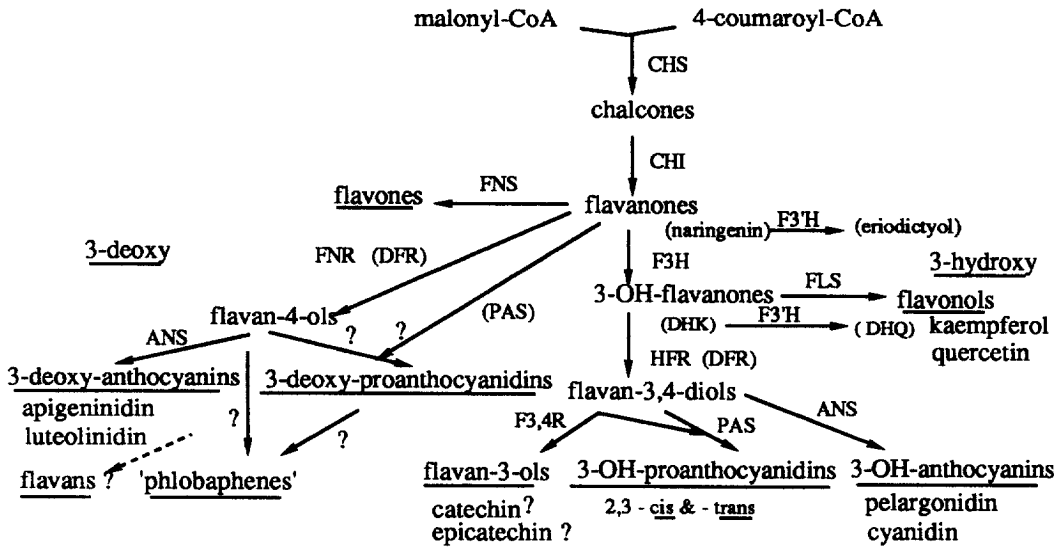

Fig. 2. Enzymes and known loci of structural genes in the C_{15} pathway to flavonoid products found in maize. CHS, chalcone synthase, at *c2* & *whp*1 loci; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3'H, flavanone 3'-hydroxylase; *pr*. *3-hydroxy pathway*: HFR (DFR), 3-hydroxyflavanone 4-reductase (dihydroflavonol reductase), *a*1; FLS, flavonol synthase; F3,4R, flavan-3,4-diol reductase (leucoanthocyanin reductase); PAS, proanthocyanidin synthase; ANS, *a*2 (anthocyanin synthase); 3-GT, 3-glucosyltransferase, *bz*1; GST, glutathione S-transferase, *bz*2; *3-deoxy pathway*: FNS, flavone synthase; FNR (DFR), flavanone reductase. (Flavan-4-ols and flavan 3,4-diols are also called leucoanthocyanidins; the sequence of 3-GT followed by GST [14] that permits the glycosylated and malonated anthocyanin to be sequestered in the central vacuole is not shown). (OH = hydroxyl group) [2–4, 22].

4-ols, 3-deoxy-anthocyanins and 3-deoxy-proanthocyanidins have been poorly characterized [8, 9]. The 3-deoxy route, leading to so-called "phlobaphenes", as insoluble mixtures, has been assayed only by the appearance of red-brown colors of unidentified 550 nm oxidation product(s) produced in strong, cold acid. Oxidized complexes of various compounds are probably involved in these insoluble components, a problem which has been discussed frequently [3, 4, 10]. Flavans have also not been identified, although this should be examined. Lastly, both flavan-4-ols and flavan-3,4-diols as well as their related oligomeric proanthocyanidins were formerly called "leucoanthocyanins", because each group gives rise to identical 3-deoxy- or 3-hydroxy-anthocyanidins when butanol-HCl mixtures are heated at near boiling temperatures. The term "leucoanthocyanidin" should now be limited to flavan-4-ols and flavan-3,4-diols [2–4]. Anthocyanins consist of both 3-deoxy and 3-hydroxy types.

FUNCTIONS OF FLAVONOIDS

Flavonols are required in anthers for functional pollen and tube growth [11]. Cold pretreatment that induces androgenesis increased the production of six flavonol glycosides, some of which may protect IAA by modulation of IAA-oxidase activity, based on cell-free experiments using these flavonols isolated from three genotypes [12]. Anthocyanins (3-hydroxy) are also present in anthers, but their function is unknown [11]. The 3-deoxy insoluble red-brown complexes known as "phlobaphenes", found in the senescent cells of the pericarp of a mature kernel in strains containing the dominant *P* allele (*P-rr*) [9], presumably serve as a physical protective outer layer and as a defense against invading microorganisms. No specific information is available for functions of flavonoids such as anthocyanins (3-hydroxy) in the aleurone layer of the endosperm, either in the mature kernel or after germination. Anthocyanin protective functions in vegetative cells under stress conditions due to UV-B damage, cadmium, abscisic acid (ABA), auxin or cold stress have recently been related to induced expression of the terminal transfer step into the vacuole by glutathione-S-transferase, which is controlled by the *bz*2 gene [13, 14]. Sheath cells with high flavonoid contents, including anthocyanins, were shown to protect maize DNA from induction of ultraviolet radiation damage by UV B and UV C [15]. Anthocyanins can also form an ascorbic acid (copigment)-metal-anthocyanin complexes that may be a protective mechanism due to detoxification of activated oxygen at stress sites as shown in rice [16]. Different types of phenolics are involved in resistance expression to pathogens in sorghum and maize [17]. Phenylpropanoids (caffeoyl esters, 3-hydroxy-anthocyanins, and lignin of unusual chemical composition (called "stress" lignin) are the important agents found in (lignin) or around (anthocyanins) infection sites in

leaves of maize, whereas 3-deoxy-anthocyanidins are the key components in sorghum pathogen resistance (found in both sites). Unlike the lignin localization, the anthocyanins (3-hydroxy) in maize, present as zwitterionic forms of a cyanidin 3-dimalonylglucoside, are not found in the infected cells, but only in epidermal cells surrounding them, possibly for removal of toxic products such as oxygen free radicals that accumulate as a result of the disease [2, 18]. These zwitterionic forms may also permit complexes with other flavonoids such as flavonols to provide UV protection.

MOLECULAR GENETICS OF MAIZE FLAVONOID PATHWAYS

Terminology problems

The molecular genetics involved in the anthocyanidin (3-hydroxy) pathway of maize have been well studied. The papers, however, suffer not only from frequently being hard to read because of varied terminology and symbols used to describe the structural and regulatory genes involved, but, most importantly, by the widespread lack of basic biochemical identifications of the flavonoid end-products presumed to be involved. The presumed anthocyanins are generally referred to only by the word "color" or "pigment", ignoring that chlorophyll, carotenoids, other phenoles etc. are also pigments. Additionally, the presence of the pericarp, the tightly bound outer layer of the one-seeded fruit or kernel of maize, is frequently ignored (without referencing or discussing the fact) that a strain with a colorless, transparent pericarp (*pp* strains) has been used, permitting visualization of the aleurone layer of the seed below. A few authors correctly describe the pericarp as being pigmented (*P-* strains) or colorless (*pp* strains) (W23, 24), or it is stated that the pericarp was removed to permit visualization and study of the aleurone layer below. The terms "white" & "colorless" for such transparent pericarps are sometimes used interchangeably, but in the case of the endodermis in edible maize kernels, the white appearance is due to total reflection of all light by the inner layer of starch. Another major problem is associated with the dominant *P* allele involved in pericarp coloration and the "plant compartment" allele P (unfortunately frequently italicized) of the *R-r* complex. The *r* locus, (sometimes listed as *r*1) involves a complex set of multiple alleles, either controlling separable seed (S), mainly in the aleurone, and (P) components of vegetative (plant) parts, or a confusing multi-tissue symbolism of a series of alleles designated as *R-r*, *R-g*, *r-r* and *r-g* [19, 20]. In the latter example, dominance or recessiveness and color in the aleurone is shown in the first term but only color for vegetative tissues in the second one. A second confusing set of multi-tissue symbols describes the multiple alleles of the *p* gene that produce either white or red pigmentation depending on the supposed presence or absence of "phlobaphenes" in the pericarp and cob, *P-rr*, *P-wr*, *P-rrw* and *P-ww* [21]. There is also inconsistency in the use of capital vs lower case for genes, loci, and alleles. In this review, I have attempted to use the lower case for genes and loci, but capitals for dominant alleles and lower case for recessive alleles; regulatory proteins are capitalized but not italicized [see ref. [20]].

Structural genes in maize

The only widely studied flavonoid pathways in maize are those that lead to flavonol glycosides, 3-hydroxy anthocyanidin glycosides and to the inadequately identified 3-deoxy "phlobaphenes" (Fig. 2). Seven of the loci of the genes or of cDNA involved have been cloned for the following enzymes: two chalcone synthases, chalcone isomerase, 3-hydroxyflavanone 4-reductase (dihydroflavonol reductase), anthocyanidin synthase, 3-glucosyltransferase, the glucosylation step followed by an acyltransferase step, and glutathione S-transferase [13], the latter enzyme permitting accumulation of anthocyanidins as malonated glycosides in the central vacuole [22], see Table 1. The structural genes, and in some cases their enzymes, in the terminal, unique steps leading to flavan-3-ols and proanthocyanidins (3-hydroxy) and to 3-deoxy anthocyanidins and possible 3-deoxy proanthocyanidins and flavans are unknown in maize (Fig. 2). The two CHS genes at *c*2 and *whp* loci are duplicated genes that are regulated differently. In the aleurone, *whp* complements *c*2 and leads to "color" formation in the presence of the homozygous recessive allele of the *intensifier* locus (*in*), but produces white pollen in sterile homozygous recessive plants (*c*2*c*2, *whpwhp*, *inin* [23, 24, 25]. The gene for chalcone synthase has been the most widely studied gene [26], and more recently that for glutathione-S-transferase that facilitates vacuolar localization of anthocyanins [13, 14].

A basic problem is whether the enzymes involved in the pathways to various flavonoid end-products at the C_{15} level in the same cell are regulated coordinately or non-coordinately, and whether channelling occurs to separate one pathway from another with common enzymes and intermediates [4, 26]. The biosynthesis of 3-deoxy- and 3-hydroxy-flavonoids in pericarp cells are controlled independently, even though there are some common enzymatic steps; there is no competition for common intermediates [9]. Different times for accumulation may be involved in complex tissues such as anthers [11]. In addition, the accumulation of flavonols found only in the tapetal layer of the anther wall was not controlled by the same genotype that controls anthocyanin formation in the epidermal layer of the anther wall. Accordingly, the problem of coordinate vs non-coordinate regulation of the entire pathway at the C_{15} level needs much research and attention, which include immunocytological techniques for

Table 1. Flavonoids identified and regulatory genes (alleles) expressed in various tissues of *Zea mays* ssp *mays* (maize); C1 Myb protein and R1 Myc protein of families of regulatory genes in parental (anthers and pericarp as floral organs) and new generation (aleurone, coleoptile, and other vegetative parts).

Plant structures, tissues	Regulatory genes (alleles) C1 family	R1 family	References
Anthers			
epidermis of wall (2n) anthocyanins	*C1*?	*R-r* complex	[11]
tapetum of wall (2n) flavonols	?	?	[11]
pollen (n) anthocyanins cyanidin 3-gly.[1]	?	*R-r* complex	[6]
"white" pollen	?	?, *inin*	[20, 24]
flavonols: quercetin, isorhamnetin kaempferol	?	*R1*	[6, 7]
Pericarp of kernel-3-deoxy-forms:			
"phlobaphenes",	*P*-complex	?	[8, 9, 34, 35]
flavan-4-ols apiferol, luteoferol	*P-rr*, *P-wr*		[8, 9, 21]
3′-deoxyanthocyanins apigeninidin, luteolinidin	*P*, *pl pl*		[8, 9]
3′-deoxyproanthocyanidins	?	?	[9]
3-hydroxy-forms:			
anthocyanin gly.[1]	*Pl*	*R1*(*R-ch*)	[8, 9, 28]
cyanidin, pelargonidin proanthocyanidins	?	?	[9]
flavones	*P*, *pl*	?	
flavonols	*Pl*	?	[8, 9]
Aleurone of kernel			
Mature kernel-			
3-deoxy anthocyanidins luteolinidin	*C1*	*R1* (*inin*)	[23]
anthocyanins (3-hydroxy) cyanidin-3-gly.[1]	*C1*	*R1* (*B-Peru*)	[19, 23, 25, 28, 32]
pelargonidin gly.[1]-red color unidentified anthocyanin	*C1*	*R1*, *in in*	[24]
ABA control-seed maturation[2]		*R-r* complex(*S*)[2]	[19, 36]
Germinated kernel-			
cyanidin-3-gly.[1]	*C1*	*R1*(*B*)	[26, 28, 36]
high light requirement	*c1-p c1-pl*	?	[28, 42]
Vegetative parts:			
Seedling-coleoptile			
3-deoxy-anthocyanidins			
luteolinidin-3-gly[1]-trace	*P-wr*	?	[5]
anthocyanidin gly.1 (3-hydroxy) cyanidin 3-gly.1-purple	?	*R-r* complex (P)[2]	[5, 19]
flavone and flavonol gly.[1]	*P-wr*?	*R-r* complex	[5, 11]
Plant-vegetative parts			
anthocyanins (3-hydroxy)	*Pl*	*R1* (*B-Peru*)	[20, 28, 33, 36]
	R-r complex (P-plant)[3]		[19]
	a3 a3[4]		[32]
variegated pattern	*Pl-Bh*	*R*(*P*)	[20, 30]
robust purple	*Pl-Rh*	*R-r*	[31]
mahogany	*Pl-mah*	*R-r*	[31]
sun-red	*plpl*	*B*	[20, 29, 30]

[1] gly.: glycosides
[2] *Vp1*: viviparous, developmental dominant allele that activates *C1* in aleurone (ABA involved + light)(Fig. 3)(36).
[3] (*P*) and (*S*): plant and seed components of *R*
[4] *a3*: intensifier with *R-g*(32).

specific enzymes and substrates to determine possible channelling and intracellular localization.

Families of regulatory genes in flavonoid pathways and tissue-specific expressions of flavonoids

Identified regulatory genes form mainly two families in maize, C1 and R1 at *c1* and *r* loci of (Table 1), and the anthocyanin pathway has been the most widely studied. Eight regulatory genes have been cloned, including the *vp1* gene that regulates the *c1* gene [22]. Alleles of both *c1* and *r1* genes are generally involved in each pathway, with multiple alleles at the same locus, especially in the case of the R family [19, 27]. The alleles of so-called "wild" strains of maize (*Zea mays*, ssp *mays*) are all dominant (although the term "wild" is never defined, but probably signifies the "control" strain used rather than an "early" maize ancestor). The *c1* locus produces Myb-homologous proteins, whereas the more complex *r1* locus produces

Myc-type proteins. The *P* allele at the *c*1 locus is responsible for "phlobaphene" red-brown color due to inadequately identified 3-deoxy compounds in the pericarp and/or cob, but it is not clear whether the dominant *P* allele acts as a single factor or requires a cofactor similar to that of the R/B and C1/Pl families [21, 28]. The dominant *Pl* allele arose as a duplication of *C*1 and leads to light-independent pigmentation in vegetative and floral organs, whereas the recessive *pl*1 alleles lead to a light-dependent sun-red pigmentation phenotype [20, 29]. Related alleles in the C1/Pl family are *Pl-Bh* that gives rise to variegated (blotched) plant tissues [30], and *Pl-Rh* and *Pl-mah* that give rise to robust purple and mahogany colors respectively [31]. The *R-r* complex of alleles with separable P and S compartments or multi-tissue symbolism (*R-r*, *R-g*, *r-r*, *r-g*), describing both aleurone, floral, and vegetative tissues as being red or green, has been widely studied [19, 20]. The *intensifier*1 allele (*in*) in the R/B family has a regulatory role affecting anthocyanin biosynthesis in the aleurone [25]. A second recessive intensifier allele, *a*3, expresses unstable purple phenotypes in vegetative tissues [32]. Paramutation, an allele-specific interaction that heritably alters transcription, has been studied for three regulatory genes, *b*, *r* of the R family and *pl* in the C1 family [33].

Regulatory genes are not expressed in all tissues or at the same time period. The problems of such tissue-specific and temporal expression of flavonoid pathways are important parts of recent research in maize. Localization of flavonoid end-products and the regulatory genes in four tissues or organs are emphasized in Table 1. The male floral organs, anthers, and the pericarp of the ovary, female floral organ, represent parental genotypes, whereas the aleurone, coleoptile and other vegetative tissues are part of the new sporophyte generation. Spatial and temporal controls in anthers are evident when the epidermis of anther walls and the tapetum (both tissues of the diploid sporophyte generation) are compared with pollen (the haploid gametophyte generation) in the biosynthesis of anthocyanins and flavonols. Anthocyanins are found only in the epidermis of the anther wall cells, whereas flavonols are synthesized in the tapetal layer of the anther wall, and are transferred to the gametophyte where they may function late in pollen development after conversion to glycosides [11].

Promoter sequences of both structural and regulatory genes are involved in regulating tissue-specific expression. The diagram in Fig. 3 is an attempt to portray recent concepts in this area for the *c*1 regulatory gene and the structural genes of the anthocyanin pathway in the aleurone layer of the kernel. The sites for attachment of R and C proteins shown in Fig. 3 are based on those identified in the promoter of the dominant *Bz*2 allele that is responsible for the vacuolar accumulation of anthocyanidin glycosides [12]. The NADPH-dependent reductase [(HFR)(DFR) or (FNR)(DFR)] steps to flavan-4-ols or 3,4-diols, which lead to either 3-deoxy or 3-hydroxy flavonoid end-products, may be catalyzed by the same enzyme controlled by the *a*1 structural gene (Fig. 2) [2, 3, 30]. The *C*1 allele together with a functional *R* or *b* allele are required to coordinately induce mRNA transcription for the biosynthesis of the anthocyanin (3-hydroxy forms) pathway in the aleurone of maize, but only the *P* allele may be required for the "phlobaphene" (insoluble 3-deoxy products in tissue extracts) in the pericarp. The mechanism for the independent regulation of these reductases by C plus B/R proteins for the *A*1 structural gene and by only the P regulatory proteins for "phlobaphenes" is of prime importance. Two binding sites for activation by C1 plus B and by P proteins, and a broader DNA binding specificity for C than for P protein may be involved [14, 34]. The promoter of the structural gene for this step common to both pathways has two C binding sites. Proteins formed by *p* and *c*1 regulatory genes bind to this same site with different affinities, but this does not explain the differential requirement for the protein from the *b* gene locus [33, 35].

Differences in the enhancer-promoter regions of regulatory alleles of the *B* gene family are also being studied to determine what sequences in the promoter

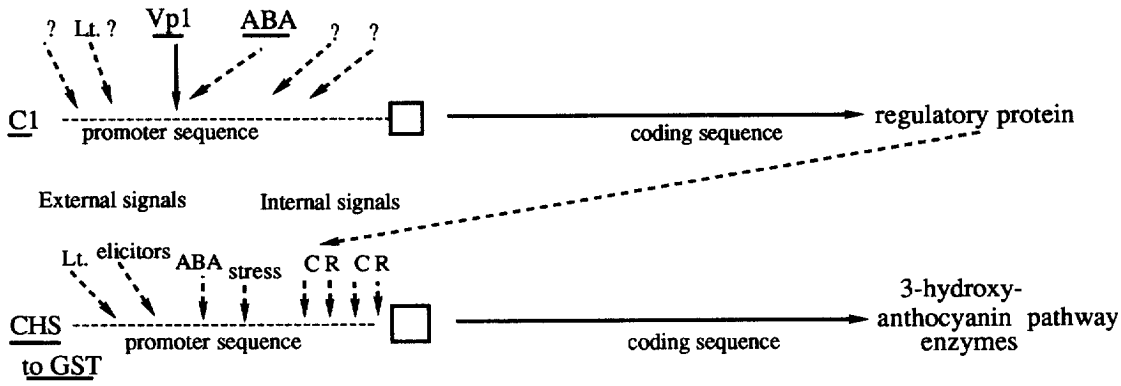

Fig. 3. An attempt to summarize possible relationships between structural and regulatory genes (alleles) in maize controlling anthocyanin biosynthesis in the aleurone during maturation of the seed [4, 26, 34, 36, 39].

are responsible for differential tissue specificity [28, 33]. The ability to cause and respond to the control of tissue specificity of anthocyanin expression by *R1* and *B1* alleles has been localized to specific portions of the promoter of the regulatory *b* gene. The protein subsequently produced by the *b* gene controls the tissue-specific expression of anthocyanin biosynthesis by attaching to specific sequences within the promoter of the structural (enzymatic) genes of the anthocyanin pathway [33].

Inducers of regulatory genes, gene hierarchy-developmental and environmental signals, light and hormones as signals

A crucial question is what regulates the regulatory alleles [26, 28, 36]. One example is the localization and synergistic interaction of the *cis*-acting elements for light, abscisic acid (ABA), and the viviparous gene (*vp1*) on the *c1* locus of maize involved in the anthocyanin pathway. Phytochrome has been shown to be a part of the light signal pathway [39]. The viviparous gene regulates maturation of seeds and other tissues. In the recessive *vp1* mutant, the absence of *C1* mRNA prevents maturation of maize seed tissues. The protein of the *Vp1* dominant allele is believed to bind upstream in the *C1* promoter and it has a role in ABA-regulated gene expression (Fig. 3) [4, 36, 39]. The *r1* gene is not regulated in the same way. The *vp1* mutant embryo has reduced sensitivity to ABA, resulting in precocious germination and blocks anthocyanin biosynthesis in the aleurone and embryo tissue. Effects of gibberellic acid and auxins in maize are still unknown. One might also question the effect of floral regulatory genes [36]. Homeotic genes that specify organ identity, including floral regulation, have been identified in *Arabidopsis* and *Antirrhinum* (MADS box), but ones controlling tissue or cell type are unknown in maize [37, 38].

Regulation of the *c1* gene by light in the aleurone layer is conditioned by signals both during maturation and germination of the seed [36]. During seed maturation, the dominant *C1* and *Vp1* alleles in combination with either an *R* or *B* dominant allele are required for the synthesis of anthocyanins in the aleurone (Table 1) [28]. Light regulation of the anthocyanin pathway can function at the levels of both the structural gene C2 and the regulatory gene *c1* (non-hierarchical), or mainly at the regulatory gene level (hierarchical) as shown in Fig. 3[39]. In addition, the presence of two recessive *c1* alleles, *c1-p* and *c1-n*, alter the interactions between light, ABA and the *vp1* gene during seed development to the mature state [36, 39, 40]. With the homozygous recessive *c1-p* allele, the aleurone is colorless upon maturation, but if light is given during seed development, the aleurone becomes pigmented only after germination in an as yet unexplained delayed reaction. A high light intensity is required, and the ratio of cyanidin to pelargonidin was lower in *c1-p* compared to the dominant *C1* allele that produces anthocyanins during maturation; the color is redder rather than purple due to the greater pelargonidin content [42]. The *vp1* gene is not required. The null *c1-n* recessive allele is not responsive to light and the aleurone remains colorless before or after germination.

The effect of both light and developmental stages on anthocyanin deposition were compared in the aleurone and pericarp layers in recent studies under field conditions [40]. The pathway in the aleurone was controlled by both *r1* and *c1* regulatory genes, whereas anthocyanin deposition within the pericarp involved the role of *Sn*, a regulatory gene in the R1 family. *Sn* expression in the pericarp, according to RNA gel blot analysis, was enhanced by light during kernel development, whereas *R* gene expression in the aleurone was not. However, husks were removed in these studies. A problem in all these studies is that only low or ill-defined levels of light may be transmitted through husk tissue of maize under field conditions [41].

In maize seedling leaf sheaths and underlying tissues, the effect of high-fluence white light on anthocyanin biosynthesis, mainly cyanidin 3-glucoside, has been reported to be regulated solely by the dominant *R* allele (the-*r* allele of the *R-r* complex) [41]. The effects of *b* or *pl* genes are negligible. However, *B pl* alleles are involved in a sun-red expression in light that occurs within 24 hours in husks, sheaths, culms, roots, and anthers (Table 1) [20, 43]. Vegetative plants with the *R-g* expression of the *R* locus are green in sunlight, but in the presence of *a3*, a recessive intensifier of plant color, dark purple sectors appear or the entire plant becomes dark purple [32].

TEOSINTE-MAIZE EVOLUTIONARY RELATIONSHIPS

There is a generally accepted hypothesis that maize was selected from annual teosintes by Native Americans in the Mexican Central Plateau more than 8000 years ago [1]. *Zea mays* ssp. *mexicano* and ssp. *parviglumus* are the most closely related wild relatives of domesticated species [43, 44]. The genome of *Zea mays* L. ($2n = 20$) is of enormous size, about twice that of mammalian genomes, with 60 to 80% repetitive information in the nuclear genome [43, 45]. Teosinte ssp. are both 2n & 4n [1]. The teosinte spike has two rows of single female spikelets that alternate between opposite sides of the rachis (cob). Each kernel is enclosed in an outer glume, the cupule of its fruitcase, and a husk [46, 47]. The first "corn", reconstructed from its oldest remains from Tehuacan, Mexico, has four ranks of paired female spikelets resulting in an 8-rowed ear. The modern ear of corn has many ranks of paired kernels that are enclosed in many husks [1].

One of the most intriguing aspects of recent genetic and developmental investigations is that the evolutionary conversion of an annual teosinte ssp (possibly *Zea mays* ssp *parviglumis*) to modern maize may have occurred by alteration of five major traits in the female inflorescence [44]. Teosinte and maize are

interfertile [46]. The most interesting trait from a physiological and biochemical point of view that needs extensive study is the *tga*1 locus [44, 47]. In an homozygous state, this locus forms a hardened encasement unit (cupulate fruitcase) in teosinte that interferes with the use of mature kernels as food, in contrast to the naked kernel on maize ears [46]. This fruitcase completely encloses the kernel so that light would not be expected to penetrate to either the pericarp or aleurone layers. The enclosure of the kernel by the cupule due to the *tgal* locus is considered one of the distinguishing steps responsible for the important characteristic of maize in prevention of self-propagation in the wild [44]. Only unidentified brown pigments are visible on the external portions of the mature fruitcase.

DNA elements associated with the region containing the *tga*1 locus have been studied by the transfer of segments of DNA containing this locus to and from teosinte and maize. When the dominant gene was transferred into teosinte, the glume tissue responsible for the cupule was reoriented so that the formerly completely encased kernel was angled out of the cupule. When *tga*1 was transferred into a colorless pericarp strain (W22 maize stock), the outer unpigmented glumes were curved upward, enlarged, and hardened (indurated) [47]. Other maize loci affecting quantitative traits (QTL) that transform both plant and inflorescence architecture in both maize and teosinte have been identified, *tb*1, *te*1 and *tru*1. A critical step was the origin of the dominant *teosinte branched*1 (*Tb*1) allele that led to apical dominance and to only short branches in modern maize, rather than to the mixture of short and long branches of teosinte precursors [46–48].

Preferences of ancient agriculturalists for visible colors probably have been responsible for the sequences involved in the activation of the anthocyanin pathway during pericarp and aleurone development in the kernel. Perhaps the highly pigmented purple, red and reddish-brown pericarps in non-agricultural maize may have been chosen for ceremonial purposes. Ears of maize for human consumption, however, were probably finally selected for their colorless, transparent pericarps and aleurone layers. The visible yellow color of the kernel in some strains is due to carotenoids, whereas the white in other cob types is due to reflection of light from the starch layer in the underlying endosperm [20].

ENZYMATIC AND REGULATORY GENES OF FLAVONOID PATHWAY IN TEOSINTE-EVOLUTIONARY ASPECTS

The basic patterns and biochemistry of pathways to flavonoids or other phenolics in teosinte are mainly unknown. None of the flavonoids found in vegetative or floral parts has been identified except by color, and the presence of other phenolics is essentially unknown. The purple compounds in vegetative tissues are assumed to be anthocyanins (3-hydroxy types) based solely on their purple color. No flavonoid pigments have been reported in the pericarp or in the aleurone until germination.

Both maize and teosinte contain dominant alleles for all enzymatic genes at the C_{15} level leading to anthocyanin glycosides in vacuoles [27]. Therefore, evolutionary changes leading to maize were accomplished by changes at regulatory loci, not enzymatic ones. Two maize loci affecting anthocyanin pathways, *c*1 and *r*1, have been studied in five different sub-species of teosinte from an evolutionary point-of-view [27]. Dominant functional alleles of enzymatic loci of teosinte were present at high frequency. However, only homozygous recessive regulatory alleles of *c*1, such as *c*1-*p* alleles, were found in most teosintes. The *c*1-*p* allele, similar to that in some lines of maize, is incapable of activating the anthocyanin pathway during kernel maturation, but it does encode functional proteins to form anthocyanins in the aleurone during germination. There is also no co-regulation by *Vp1* alleles and ABA to permit development of a purple aleurone upon maturation of the seed as in maize [36, 38]. The authors argue that since functional C1 protein is found in the aleurone only after germination, the lack of expression in the aleurone during kernel maturation was due to differences in *cis*-regulatory elements at this regulatory locus. Evolution to maize would require changes in these cis-regulatory elements [27]. The *r* locus in teosinte has been less well studied, but the recessive *r* (*r-r*) allele is considered to be present in teosinte, since the red anthocyanin color found only in vegetative tissues indicates that a functional R protein must be present [27]. Changes in *cis*-regulatory elements in the promoter at the *r*1 regulatory locus would be necessary for evolution to maize strains. A small portion of the *r* gene from teosinte has been cloned [19].

Critical biochemical identifications of phenolics in teosinte are vital. In terms of the evolution of teosinte to maize, changes in the control of anthocyanin biosynthesis by light becomes critical. The origin of the brown colors of the hardened fruitcase enclosing the kernel is unknown, and oxidation of phenolic compounds would be expected. Since the recessive *c*1-*p* allele described in a previous section is present in some teosintes [27], the effect of light should be studied in teosinte not only in terms of effects upon germination, but also during the maturation stages of the kernels in order to determine whether the potential for a delayed reaction is present if light is experimentally introduced briefly through an opening.

MAJOR GOALS FOR FUTURE RESEARCH IN TEOSINTE AND MAIZE FOR BIOCHEMISTS, ENZYMOLOGISTS AND PHYSIOLOGISTS

Whereas geneticists will continue to study the structural and regulatory genes involved, cooperation with plant biochemists, enzymologists and physiologists is

necessary if the molecular genetics is to be understood-this should be a challenging area in both maize and teosinte. Identification of all the flavonoids shown in Fig. 2 is critical for both teosinte and maize. Only anthocyanins and flavonols (both in the 3-hydroxy pathway) have been adequately identified chromatographically. For instance, the various compounds listed in Table 1 and in the text, reported only by color such as red, purple, red-brown, brown, robust purple, sun-red, and mahogany, have never been isolated and identified chemically or chromatographically. Mixtures of phenolic compounds could be involved in mature tissues. The 3-deoxy end-products, the insoluble oxidized products called "phlobaphenes", 3-deoxy anthocyanins and pro-anthocyanidins, have been inadequately identified. The methanol or aqueous insoluble fractions must be studied by techniques other than HPLC columns that demand initial purification of crude extracts. Developmental studies are needed to identify flavonoids and various brown-black colors prior to senescence in both maize and teosinte.

What regulates the acetate and phenylpropanoid pathways that provide the two substrates required by chalcone synthase? Competition with other pathways using the same compounds must be involved. At the C_{15} level starting with chalcone synthase, are the enzymic pathways using the same intermediates in one cell regulated coordinately as a single pathway? Coordinate control of anthocyanin gene expression requires that defined promoter sequences in all of the structural genes of the anthocyanin pathway interact with C1 and R1 family members in a similar way [26]. If control is non-coordinate, how is the competition for intermediates regulated? While the end-products such as anthocyanins accumulate in large or small vacuoles, an immuno-histochemical approach is necessary to determine just where the intermediates and enzymes involved in flavonoid pathways are found in different parts of the cell. Are these intermediates free in the cytoplasm or enclosed in vesicles such as cyanoplasts that are budded off from the ER? [4]. The wall area cannot be excluded. Physiological experiments in teosinte to determine the wavelengths of light, photoreceptors and the interactions with hormones such as ABA that are involved in the formation of flavonoids will be critical in understanding the evolutionary relationships between teosinte and maize. Can any present maize line be identified as the best example of a primitive form?

A basic gap in both maize and teosinte biology exists!

REFERENCES

1. Galinat, W. C., in *Corn and Corn Improvement*. American Society of Agronomy Monograph no. 18, 3rd. edition, eds G. F. Sprague and J. W. Dudley, 1988, pp. 1–31.
2. Heller, W. and Forkmann, G., in *The Flavonoids, Advances of Research Since* 1986, ed. J. B. Harborne, 1994, pp. 499–535.
3. Stafford, H. A., in *Polyphenolic Phenomena*, ed. A. Scalbert. INRA Editions, Paris, France, 1993, 73–80.
4. Stafford, H. A., An American Society of Plant Physiologists Series, *Current Topics in Plant Physiology*, 1995, **15**, 15.
5. Styles, E. D. and Ceska, O. S., *Canadian Journal of Genetics Cytology*, 1981, **23**, 691.
6. Styles, E. D. and Ceska, O., *Maydica*, 1981, **26**, 141.
7. Ceska, O. and Styles, E. D., *Phytochemistry*, 1984, **23**, 1822.
8. Styles, E. D. and Ceska, O., *Canadian Journal of Genetics Cytology*, 1977, **19**, 289.
9. Styles, E. D. and Ceska, O., *Maydica*, 1989, **34**, 227.
10. Stafford, H. A., in *Discoveries in Plant Biology*, World Science Publications, Singapore, 1998, 345–361.
11. Deboo, G. B., Albertsen, M. C. and Taylor, L. P., *Plant Journal*, 1995, **7**, 703.
12. Delalonde, M., Barret, Y. and Coumans, M. P., *J. Plant Physiol.*, 1996, **149**, 612.
13. Marrs, K. A., Alfenito, M. R., Lloyd, A. M. and Walbot, V., *Nature*, 1995, **375**, 397.
14. Marrs, K. A. and Walbot, V., *Plant Physiology*, 1997, **113**, 93.
15. Stapleton, A. E. and Walbot, V., *Plant Physiology*, 1994, **105**, 881. 1997, **20**, 279.
16. Sarma, A. D., Sreelakshmi, Y. and Sharma, R., *Phytochemistry*, 1997, **45**, 671.
17. Nicholson, R. L. and Hipskind, J. D., in *Proceedings of the Japan-US scientific Seminar on Molecular Aspects of Pathogenicity and Host Resistance: Requirements for Signal Transduction*, eds D. Mills, H. Kunoh, N. T. Keen, S. Mayamma. APS Press, 1996, pp. 209–217.
18. Hipskind, J., Wood, K. and Nicholson, R. L., *Physiological and Molecular Plant Pathology*, 1996, **49**, 247.
19. Walker, E. L., Robbins, T. P., Bureau, T. E., Kermickle, J. and Dellaporta, S. L., *Embo Journal*, 1995, **14**, 2350.
20. Neuffer, M. G., Coe, E. H. and Wessler, S. R., in *Mutants of Maize*. Cold Spring Harbor Laboratory Press, 1997, p. 468.
21. Chopra, S., Athma, P. and Peterson, T., *Plant Cell*, 1996, **8**, 1149.
22. Holton, T. A. and Cornish, E. C., *Plant Cell*, 1995, **7**, 1071.
23. Reddy, A. R. and Peterson, P. A., *Canadian Journal of Genetics and Cytology*, 1978, **20**, 337.
24. Franken, P., Niesbach-Klösgen, U., Weydemann, U., Maréchal-Drouard, S. and Wienand, U., *The EMBO Journal*, 1991, **10**, 2605.
25. Burr, F. A., Burr, B., Scheffler, B. E., Blewitt, W., Wienand, U. and Matz, E. C., *Plant Cell*, 1996, **8**, 1249.

26. Mol, J. N. M., in *Polyphenolic Phenomena*, ed. A. Scalbert, 1993, pp. 87–98.
27. Hanson, M. A., Gaut, B. S., Stec, A. O., Fuerstenberg, S. I., Goodman, M. M., Coe, E. H. and Doebley, J. F., *Genetics*, 1996, **143**, 1395.
28. Dooner, H. K., Robbins, T. R. and Jorgenson, R. A., *Annual Review of Genetics*, 1991, **25**, 173.
29. Cone, K. C., Cocciolone, S. M., Moehlenkamp, C. A., Weber, T., Drummond, B. J., Tagliani, L. A., Bowen, B. A. and Perrot, G. H., *Plant Cell*, 1993, **5**, 1807.
30. Cocciolone, S. M. and Cone, K. C., *Genetics*, 1993, **135**, 575.
31. Hollick, J. B., Patterson, G. I., Coe, E. H. Jr., Cone, K. C. and Chandler, V. L., *Genetics*, 1995, **141**, 709.
32. Styles, E. and Coe, E. H. Jr., *Journal of Heredity*, 1986, **77**, 389.
33. Patterson, G. I., Kubo, K. M., Shroyer, T. and Chandler, V. L., *Genetics*, 1995, **140**, 138.
34. Sainz, M. B., Grotewold, E. and Chandler, V. L., *The Plant Cell*, 1997, **9**, 611.
35. Grotewold, E., Drummond, B. J., Bowen, B. and Peterson, T., *Cell*, 1994, **76**, 543.
36. McCarty, D. R., *Annual Review Plant Pysiology And Plant Moleclular Biology*, 1995, **46**, 71.
37. Ma, H., *Cell*, 1997, **89**, 821.
38. Doebley, J., *Current Opinion in Genetics and Development*, 1993, **3**, 865.
39. Kao, C., Cocciolone, S. M., Vasil, I. K. and McCarty, D. R., *Plant Cell*, 1996, **8**, 1171.
40. Procissi, A., Dolfini, S., Ronchi, A. and Tonelli, C., *Plant Cell*, 1997, **9**, 1547.
41. Taylor, L. P. and Briggs, W. R., *Plant Cell*, 1990, **2**, 115.
42. Chen, S.-M. and Coe, E. H. Jr., *Biochem. Genetics*, 1977, **15**, 333.
43. Coe, E. H., Jr., Neuffer, M. G. and Hosington, D. A., in *Corn and Corn Improvement*. Agronomy Monograph no. 18, 3rd. edition, eds G. E. Sprague and J. W. Dudley, 1988, pp. 81–258.
44. Szabó, V. M. and Burr, B., *Molecular Genetics and Genetics*, 1996, **252**, 33.
45. Walbot, V. and Messing, J., in *Corn and Corn Improvement*. Agronomy Monograph no. 18, 3rd. edition, 1988, pp. 389–429.
46. Doebley, J. and Stec, A., Gustus, C., *Genetics*, 1995, **141**, 333.
47. Dorweiler, J., Stec, A., Kermicle, J. and Doebley, J., *Science*, 1993, **262**, 233.
48. Doebley, J., Stec, A. and Hubbard, L., *Nature*, 1997, **386**, 485.