Regulation of Lysine and Threonine Synthesis

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INTRODUCTION

Human and monogastric animals cannot synthesize 10 out of the 20 amino acids and therefore need to obtain these from their diet. Among the essential amino acids, lysine and threonine are considered to be exceedingly important in that they are the most limiting essential amino acids in cereal grains, which represent the largest source of food worldwide (Bright and Shewry, 1983). Because of the nutritional importance of lysine and threonine, the regulation of their metabolism has been studied extensively at the biochemical, genetic, and, more recently, molecular levels. Like many bacterial species, higher plants synthesize lysine and threonine from aspartate using two different branches of the aspartate family pathway, as shown schematically in Figure 1. The enzymes involved in lysine and threonine synthesis have been reviewed in detail (Bryan, 1980). In this review, I discuss the complex biochemical, cellular, developmental, physiological, and environmental controls of the synthesis of lysine and threonine. I also focus on specific enzymes that play major regulatory roles in the synthesis of these amino acids.

BIOCHEMICAL REGULATION OF LYSINE AND THREONINE SYNTHESIS

Analyses of crude and purified enzyme preparations have shown that the aspartate family pathway in higher plants is regulated by several end product feedback inhibition loops (Figure 1, curved arrows). Similar feedback inhibition loops also operate in many bacterial species (Cohen and Saint-Girons, 1987). The activity of aspartate kinase (AK), the first enzyme in the pathway, is feedback inhibited by both lysine and threonine. In addition, lysine inhibits the activity of dihydricolinate synthase (DHPS), whereas threonine inhibits the activity of homoserine dehydrogenase (HSD). Therefore, in addition to feedback inhibiting the first enzyme in the pathway, lysine and threonine also inhibit the activities of the first enzymes after the branch point, that is, enzymes that are specific for their own synthesis (Figure 1).
The major regulatory enzymes and their products are indicated on a transmission electron micrograph of a plastid. The pathway from threonine to isoleucine is not shown. Yellow curved arrows represent feedback inhibition by the end product amino acids. AK, aspartate kinase; DHPS, dihydrodipicolinate synthase; HSD, homoserine dehydrogenase. The branches of lysine and threonine syntheses appear to be localized in the plastid, whereas methionine appears to be synthesized in the cytosol (for review, see Bryan, 1990).

lysine-sensitive AK isozyme that is similar to the E. coli lysinesensitive AK-III has been cloned from plants, and it is thus still not known whether plants also contain an AK isozyme that lacks HSD activity, as is the case with E. coli AK-III (Cohen and Saint-Girons, 1987).

**Dihydrodipicolinate Synthase**

DHPS catalyzes the first reaction that is unique to lysine biosynthesis, the condensation of 3-aspartic semialdehyde with pyruvate to form 2,3-dihydrodipicolinate. Among the major regulatory enzymes of the aspartate family pathway in plants, DHPS is the most sensitive to feedback inhibition by lysine ($I_{0.5}$ between 10 and 50 μM). DHPS is ~10-fold more sensitive to lysine inhibition than are plant lysine-sensitive AKs ($I_{0.5}$ between 100 and 700 μM) and ~100-fold more sensitive to lysine inhibition than is E. coli DHPS ($I_{0.5}$ of ~1000 μM). DHPS has been purified to near homogeneity from wheat (Kumpaisal et al., 1987), tobacco (Ghislain et al., 1990), maize (Frisch et al., 1991), and pea (Dereppe et al., 1992), and DHPS cDNAs and genes have been cloned from several plant species (Kaneko et al., 1990; Frisch et al., 1991; Vauterin and Jacobs, 1994). Results of DNA gel blot analysis suggest that maize may contain several DHPS genes (Frisch et al., 1991). Similarly, two different DHPS cDNAs have been cloned from common wheat (Kaneko et al., 1990).

**Homoserine Dehydrogenase**

HSD catalyzes the first reaction that is uniquely associated with threonine, methionine, and isoleucine biosynthesis, the conversion of 3-aspartic semialdehyde into homoserine. Higher plants generally possess at least two forms of HSD—a threonine-sensitive form and an insensitive form (Bryan, 1980; Lea et al., 1985). HSD has been purified to near homogeneity from a variety of plant species. Carrot HSD can be reversibly interconverted in vitro from a threonine-sensitive trimeric form to a threonine-insensitive dimeric form, depending on whether the medium contains threonine (Matthews et al., 1989). It is still not known whether this interconversion occurs in vivo and, if so, whether it helps regulate threonine synthesis. Characterization of purified HSD (Wilson et al., 1991; Azevedo et al., 1992) and cDNA clones (Weisemann and Matthews, 1993; Ghislain et al., 1994; Muehlbauer et al., 1995) has confirmed that the HSD isozymes are linked to AK on single proteins.

**Intracellular Localization of the Aspartate Family Pathway**

Many of the enzymes involved in lysine and threonine synthesis have been localized to plastids (Bryan, 1990). Analyses of cloned DNA sequences confirm that these enzymes are
synthesized with transit peptides that direct them into the plastid (Kaneko et al., 1990; Frisch et al., 1991; Weisemann and Matthews, 1993; Ghislain et al., 1994; Muehlbauer et al., 1995). Operation of the aspartate family pathway inside the plastid makes biological sense because some of the biochemical reactions require energy that is largely produced within this organelle. Nevertheless, some of the aspartate family enzymes, particularly those involved in the synthesis of methionine and S-adenosyl methionine, have been localized to the cytosol (Bryan, 1990). Several functional and regulatory aspects of the intracellular localization of the aspartate family enzymes are discussed in later sections.

Functional Studies of the Aspartate Family Pathway

Although in vitro studies of enzyme activities have pinpointed the major regulatory enzymes of the aspartate family pathway, they have not provided sufficient evidence regarding the exact functional roles of each of these enzymes. Nor have these studies shown how these major regulatory enzymes operate in concert to regulate this complex pathway. Functional evidence of this regulation has been obtained by analyzing plants possessing feedback-insensitive forms of the major regulatory enzymes. These plants include both mutant strains and transgenic plants that overexpress bacterial genes encoding several major regulatory enzymes of the aspartate family pathway. The results of these studies have also highlighted important regulatory differences between the aspartate family pathways of higher plants and bacteria.

Analyses of Plant Mutants

In E. coli, the rate-limiting enzyme for lysine biosynthesis is AK, with DHPS playing only a secondary role. This was shown by the analysis of E. coli mutants selected for their resistance to inhibition by toxic lysine analogs such as thiosine or S-aminoethyl L-cysteine (AEC). These analogs compete with lysine for incorporation into proteins, and only mutants that overproduce lysine survive such treatment (Bright and Shewry, 1983; Jacobs et al., 1987). The E. coli lysine-overproducing mutants selected on this basis possess a modified, lysine-insensitive form of the AK-III isozyme (Boy and Patte, 1972). This suggests that feedback inhibition of AK by lysine is the main point of regulation for lysine biosynthesis in E. coli (Cohen and Saint-Girons, 1987). Because the bacterial mutants overproduce lysine but not threonine, it is likely that inhibition of HSD by threonine is the main point of regulation for threonine biosynthesis (Figure 1; Cohen and Saint-Girons, 1987). Any physiological role of DHPS inhibition by lysine in E. coli is questionable, because mutants with feedback-insensitive DHPS have not been found, despite the use of strong selective agents (Cohen and Saint-Girons, 1987). This is in accord with the fact that the E. coli AK-III is considerably more sensitive to lysine inhibition than is E. coli DHPS (\(I_{0.5} = 0.2\) mM and 1 mM, respectively).

Similar selections for AEC-resistant, lysine-overproducing plant mutants have been successful for several species, including carrot, tobacco, rice, and bulrush millet (for review, see Galili et al., 1994, and references therein). The AEC resistance in tobacco cells resulted from the expression of a modified, less lysine-sensitive DHPS (Negrutiu et al., 1984). This indicates that in plants, in contrast with E. coli, DHPS is the major rate-limiting enzyme in lysine biosynthesis and AK plays a secondary role. Indeed, plant DHPSs are generally 20 to 100 times more sensitive to lysine inhibition than is E. coli DHPS.

Plants containing feedback-insensitive AK isozymes have, however, been identified by selecting for resistance to growth inhibition by high concentrations of lysine plus threonine. These two amino acids together completely inhibit the activity of all AK isozymes, resulting in methionine starvation (Figure 1; Green and Phillips, 1974; Jacobs et al., 1987). Mutants possessing feedback-insensitive AK isozymes have been selected in a number of plant species by their resistance to growth on lysine plus threonine (for review, see Galili et al., 1994). These mutants overproduce free threonine and, to a lesser extent, isoleucine, methionine, and, in some cases, lysine. These results show that in plants, in contrast to bacteria, AK limits mainly threonine but not lysine synthesis. Indeed, HSD activity could play only a secondary role in limiting threonine synthesis in plants, because in contrast to bacteria, plants contain at least one HSD isozyme that is insensitive to feedback inhibition.

Analyses of Transgenic Plants

Although plant mutants have contributed to our understanding of the regulation of lysine and threonine synthesis, such mutants have several major limitations because of the selection procedures by which they are obtained. Because the sensitivities of AK and DHPS to feedback inhibition are the major limiting factors for lysine and threonine synthesis, mutants isolated for resistance to lysine plus threonine or to AEC possess structurally modified enzymes rather than enzymes with altered expression or compartmentation. Such mutants are therefore not useful for understanding regulation by the levels, intracellular localization, or tissue-specific expression of the enzymes. These limitations have been overcome by expressing bacterial genes encoding feedback-insensitive AK and DHPS enzymes in transgenic plants (Glassman et al., 1992; Perl et al., 1992; Shaul and Galili, 1992a, 1992b).

To create such transgenic plants, the coding sequences of the bacterial AK and DHPS genes have been fused to the constitutive 35S promoter from cauliflower mosaic virus. Some chimeric constructs have also included DNA encoding a plastid transit peptide to direct the bacterial enzymes into the organelle. Expression of the bacterial AK results in significant overproduction of free threonine, whereas expression of the bacterial
DHPS causes significant overproduction of lysine (Glassman, 1992; Perl et al., 1992; Shaul and Galili, 1992a, 1992b), in agreement with studies of plant mutants.

In addition, the transgenic plants have led to important additional discoveries. First, they have shown that localization of DHPS in the plastid is crucial for lysine synthesis, whereas localization of AK within the organelle improves threonine production but is not essential (Shaul and Galili, 1992a, 1992b). The biological significance of this observation is not clear because it is still unknown whether all plant AK isozymes are localized in plastids. It is possible that some metabolic intermediates of the aspartate family pathway can shuttle between the plastids and cytosol. Studies of transgenic plants have also demonstrated not only that AK and DHPS are regulated by feedback inhibition but also that the levels of these enzymes limit the rate of production of threonine and lysine. When the entire green tissue from 1-month-old plantlets derived from transgenic plants possessing various levels of the bacterial enzymes was analyzed, significant positive correlations were detected between the levels of the bacterial AK and DHPS enzymes and the levels of free threonine and lysine, respectively (Shaul and Galili, 1992a, 1992b). Thus, the biochemical regulation of threonine and lysine synthesis apparently operates on both the Km and Vmax of AK and DHPS. Finally, the lysine and threonine levels of the transgenic plants vary in different tissues and at different stages of development, suggesting the presence of complex developmental regulatory signals leading to differential expression of aspartate family genes.

Concerted Regulation of Lysine and Threonine Synthesis

Transgenic plants expressing both a feedback-insensitive AK and a feedback-insensitive DHPS (Frankard et al., 1992; Shaul and Galili, 1993) contain free lysine levels that far exceed those in plants expressing only the insensitive DHPS or AK. This lysine increase is also accompanied by a significant reduction in threonine accumulation as compared with plants expressing the insensitive AK only. These results reveal two major aspects of the regulation of the aspartate family pathway. First, the balance between lysine and threonine synthesis appears to be determined by a competition between DHPS and HSD for limiting amounts of their common substrate, 3-aspartic semialdehyde, whose level is apparently determined primarily by the activity of AK (see Figure 1). This agrees with the fact that 3-aspartic semialdehyde is a very unstable compound in vitro and that the level of aspartate, the precursor of the aspartate family pathway, has never been found to be limiting (Frankard et al., 1991, 1992; Perl et al., 1992; Shaul and Galili, 1992a, 1992b, 1993). Second, among the aspartate family amino acids in plants, lysine synthesis appears to be under the most stringent regulation because DHPS is so sensitive to lysine inhibition. When DHPS becomes deregulated, the branch leading to lysine synthesis competes strongly with the other branch of the pathway, and a considerable amount of 3-aspartic semialdehyde is thus converted into lysine at the expense of threonine.

DEVELOPMENTAL AND ENVIRONMENTAL REGULATION OF LYSINE AND THREONINE SYNTHESIS

Are Multiple AK Isozymes Redundant or Essential?

Although the aspartate family pathway has been studied mostly at the biochemical level, several lines of evidence suggest that this pathway is also subject to complex developmental and environmental regulation. For instance, AK, the first enzyme of the aspartate family pathway, apparently has major regulatory importance for the synthesis of all of the aspartate family amino acids. Close examination of the aspartate family pathway from a biochemical viewpoint suggests that despite the major regulatory importance of AK, feedback inhibition of the lysine-sensitive AK isozymes by lysine itself is unlikely to play a major regulatory role. DHPS is much more sensitive to lysine inhibition than is AK, and lysine levels should therefore never accumulate enough to inhibit AK activity. If the level of lysine-sensitive AK activity is not limiting, then the threonine-sensitive AK isozyme may be biochemically redundant, as is the case in E. coli (Cohen and Saint-Girons, 1987). On the other hand, if different AK isozymes are expressed in cell- and tissue-specific manners, then the different AK isozymes may not be redundant.

Indeed, several lines of evidence suggest that there is no absolute redundancy of AK activities in plants and that most if not all of the AK isozymes are functional and essential. First, although joint addition of lysine and threonine strongly inhibits plant growth, each of these amino acids by itself can inhibit growth when added alone at higher concentrations (Bryan, 1980). Moreover, the sensitivity of plant growth to either lysine or threonine varies markedly at different stages of development (H. Karchi, L. Yang, R. Amir, and G. Galili, unpublished results). These findings imply that both the lysine- and threonine-sensitive AKs are essential for plant growth. Second, the relative activities of lysine- and threonine-sensitive AKs change dramatically during plant development, with the lysine-sensitive activity dominating in rapidly growing cells (Bryan, 1980, 1990). In addition, the levels of AK and DHPS mRNAs are higher in young leaves than in elongating leaves (X.-Z. Zhu and G. Galili, unpublished observations). Third, lysine-sensitive AK should be only weakly affected by lysine because of the extreme sensitivity of DHPS to lysine inhibition (see Figure 1). This is not the case in vivo; mutations rendering plant lysine-sensitive AKs insensitive to lysine cause overproduction of threonine (see Galili et al., 1994, and references therein).

Moreover, although the lysine-sensitive AKs appear to be subjected to regulation in vivo, they still do not limit lysine synthesis. In fact, the mechanism of AK regulation is even more complex. So far, all of the threonine-overproducing plant
mutants have been shown to possess a modified, lysine-insensitive form of the lysine-sensitive AK but not of the threonine-sensitive AK/HSD enzymes (see Galili et al., 1994, and references therein). The reason for this is still unknown, but it may result from different mechanisms of allosteric inhibition of the lysine- and threonine-sensitive AKs. Indeed, a recent report (Muehlbauer et al., 1995) suggested that the allosteric regulation of the threonine-sensitive AK/HSD enzymes of maize (and perhaps of other plants as well) may occur by a novel, two-component mechanism in which the AK domain acts as a sensor of the threonine concentration by autophosphorylation of a histidine residue in this domain. The phosphate is then transferred from the sensor to a specific aspartic acid residue that may be located in the HSD domain to initiate a response regulatory program. This hypothesis is based on amino acid similarities of the maize AK/HSD enzymes to other proteins regulated by an analogous two-component mechanism.

Lysine Synthesis during Plant Development Is Not Dependent Solely on DHPS Activity

Despite the key regulatory role of DHPS, lysine production also depends on additional enzymes. Expression of a feedback-insensitive DHPS should elevate lysine synthesis only in cells in which the entire lysine biosynthetic pathway is active. The levels of free lysine in young tobacco leaves are significantly higher than those in older leaves, even though younger leaves have lower expression of the bacterial DHPS (O. Shaul and G. Galili, unpublished results). This observation is in accord with the findings that the aspartate family pathway, as a whole, is more active in rapidly growing young leaves than in old leaves (X.-Z. Zhu and G. Galili, unpublished data).

Regulation of Lysine and Threonine Synthesis during Seed Development

The major amino acids transported into seeds are asparagine and/or glutamine. Seeds are thus expected to possess the entire enzymatic machinery needed to convert asparagine and glutamine into the other amino acids. To test whether seeds have the potential to synthesize lysine and threonine autonomously, transgenic tobacco plants were created that express bacterial feedback-insensitive AK and DHPS under the control of the seed-specific promoter from the bean phaseolin storage protein gene (Karchi et al., 1993, 1994). Seed-specific expression of the bacterial feedback-insensitive AK resulted in a significant increase in free threonine in mature seeds. Similarly, plants expressing the bacterial feedback-insensitive DHPS in a seed-specific manner synthesized higher than normal levels of free lysine during seed development. However, the level of free lysine was significantly reduced in mature seeds, most likely because it is efficiently catabolized (Karchi et al., 1994). These results indicate that the aspartate family pathway is functional in seeds.

The timing of expression of the lysine and threonine biosynthesis genes as well as the metabolism of asparagine into aspartate and aspartate family amino acids seems to occur relatively late during seed development, similar to the timing of expression of storage protein genes (Karchi et al., 1994). These observations suggest that genes encoding storage proteins as well as genes encoding enzymes involved in amino acid metabolism are regulated coordinately during seed development. If there is indeed a coordinated regulation of genes encoding storage proteins and metabolic enzymes, one might expect to find common promoter elements in them. Although detailed studies of the promoters of genes encoding amino acid biosynthesis enzymes are still lacking, it appears that they may contain at least some promoter elements that are similar to promoter elements of storage protein genes (see the following section).

Environmental Control of Lysine and Threonine Synthesis

The production of lysine and many other amino acids in yeast is coordinately regulated by nutritional conditions. This regulation is termed the general control (GCN) system. The GCN4 transcription factor, which binds to specific short conserved regions in the promoters of many genes encoding enzymes of amino acid metabolism, is a major determinant of this regulation (Hinnebusch, 1988). Many plant genes contain GCN4-like boxes in their promoters (Müller and Knudsen, 1993). These genes encode not only enzymes of amino acid metabolism but also storage proteins and a few enzymes that are not involved in amino acid metabolism. This further suggests that amino acid metabolism and storage protein synthesis are coordinately regulated during seed development. Interestingly, the Opaque-2 transcription factor of maize, which is an important regulator of storage protein synthesis, binds to promoters of GCN4-activated yeast genes and can also complement yeast mutants lacking GCN4 activity (Mauri et al., 1993). The functional significance of the GCN4-like boxes in regulating gene expression in seeds and nonseed tissues is still not understood. It has, however, been demonstrated that the GCN4-like box might be a key element in regulating the response of a barley storage protein promoter to nitrogen (Müller and Knudsen, 1993).

LYSINE CATABOLISM

Lysine catabolism has been studied extensively in microorganisms, fungi, and animals. In plants, lysine catabolism has been studied mostly in seeds. In cereal and legume seeds, lysine is catabolized via saccharopine and α-amino acid semialdehyde, the same pathway as in mammalian cells (Figure 2; Mazelis, 1980). The first reaction in this pathway is the conversion of lysine plus α-ketoglutarate into saccharopine,
subject to complex developmental and environmental regulation, developing maize grains and tobacco seeds appears coordinately with the onset of storage protein synthesis (Brochetto-Braga et al., 1992; Karchi et al., 1994), and this activity is noticeably lower in developing grains of opaque-2 maize mutants than in wild-type grains (Brochetto-Braga et al., 1992). Increasing the concentration of free lysine in developing tobacco seeds, either by exogenous administration or by endogenous overproduction (via expression of the bacterial feedback-insensitive DHPS), causes an ~10-fold increase in the activity of lysine ketoglutarate reductase (Karchi et al., 1994). Thus, free lysine accumulation, and possibly its incorporation into seed proteins, is apparently a highly regulated process.

**FUTURE PROSPECTS**

**Molecular and Cellular Dissection**

Cloned genes for aspartate family enzymes help in elucidating the cellular and developmental regulation of expression of these genes as well as the in situ localization of their mRNAs and proteins. Several of these genes have been cloned from different plant species, among them genes that appear to encode threonine-sensitive AK/HSD enzymes and others that encode DHPS. Presumably, additional genes encoding aspartate family enzymes will soon be cloned. Cloning of the lysine-sensitive AK is of particular importance, because it will provide a critical tool to address the question of the lack of redundancy of AK isozymes in plants.

It will also be important to perform functional analyses of promoters driving the expression of aspartate family genes and to identify transcription factors and other proteins that bind to these promoters. These types of studies may help in elucidating general control mechanisms as well as in identifying mechanisms involved in the regulation of lysine and threonine synthesis. The available physiological data indicate that the promoters of the aspartate family genes are likely to be complex. These promoters are likely to contain several regulatory sequences that are also present in storage protein genes, such as the Opaque-2 box. If the aspartate family enzymes in seeds and other vegetative tissues are encoded by common genes, these genes may possess additional controlling elements that are different from those of storage protein genes and that regulate their expression in tissues other than seeds. Several putative regulatory elements have already been identified in the promoters of aspartate family enzymes by sequence homology (Ghislain et al., 1994), but their functional significance has still to be determined.

The intracellular localization of the aspartate family enzymes and its regulatory consequence also need to be examined. Many of the aspartate family enzymes have been localized to plastids, but it is still not clear whether this is true for all enzymes. This question is particularly relevant for the different AK isozymes, because AK has been observed to function in both the plastid and the cytosol (Shaul and Galili, 1992b). The cloning of the entire set of AK isozymes as well as the production of isozyme-specific antisera based on amino acid sequence variations between the sequences will be required to address fully the question of AK localization.

**Genetic Dissection**

Genetic identification and subsequent cloning of new structural and regulatory genes in the aspartate family pathway are also called for. Two genetic approaches can be used: (1) the production of auxotrophs that cannot synthesize lysine and threonine; and (2) the identification of regulatory mutants in which the synthesis and final accumulation of lysine and threonine are enhanced or suppressed. Auxotrophs may be obtained by employing negative complementation (Last, 1993) using cloned genes or by selecting plants that require aspartate family amino acids for growth. Selections for regulatory mutants can be performed either by classical mutagenesis or by more recently developed approaches such as insertion mutagenesis, transposon mutagenesis, or activation of genes by transforming plants with strong enhancers (Hayashi et al., 1992). However, selection for regulatory mutants of the aspartate family pathway is not simple. Because lysine and threonine synthesis is regulated primarily by feedback inhibition of DHPS and AK, mutants selected for resistance to toxic lysine and threonine analogs generally result in mutations in the coding sequences of these enzymes that render them feedback insensitive.
because overproduction of lysine or threonine causes severely insensitive rather than in mutations in regulatory genes. Nevertheless, genetic dissections may be aided significantly because overproduction of lysine or threonine causes severely abnormal plant phenotypes (Frankard et al., 1992; Shaul and Galili, 1993) and lysine overproduction renders plants extremely sensitive to exogenous threonine (Perl et al., 1992; Shaul and Galili, 1992a). The latter effect of lysine overproduction results because the endogenously overproduced lysine and the exogenously added threonine significantly inhibit AK, causing starvation for methionine (Figure 1). Mutagenized plants could thus be screened for the enhancement or suppression of the abnormal phenotypes as well as for suppression of the threonine sensitivity. Indeed, we have recently obtained putative mutants with suppressed threonine sensitivity (H. Karchi, L. Yang, and G. Galili, unpublished data). These putative mutants may result from any of several defects, such as defective lysine synthesis, increased lysine catabolism, increased accumulation of soluble methionine, or altered lysine sequestration.

Environmental and Physiological Controls

Lysine and threonine synthesis requires carbon, nitrogen, and energy. Little is known about the interrelationships among these factors, and it will be important to study how lysine and threonine synthesis is regulated by carbon and nitrogen flows as well as by energy obtained from photosynthesis. Again, the abnormal phenotypes associated with lysine and threonine overproduction will be invaluable in unraveling these questions. We have recently found that transgenic lysine-overproducing tobacco plants, when grown under relatively low light intensity (50 μE m⁻² sec⁻¹), lose much of their morphological abnormalities and accumulate much lower lysine levels than plants grown at 200 μE m⁻² sec⁻¹. Whether this is due to reduced energy available from photosynthesis or to light-regulated expression of aspartate family genes remains to be determined. A similar approach could be used to test how lysine and threonine synthesis is regulated by photosynthesis and by various plant hormones.

Practical Implications and Applications

The developments described previously have the potential to lead to the production of more nutritious transgenic crop plants. Targeting overproduction of free lysine and threonine to specific tissues, such as seed, can eliminate problems associated with abnormal phenotypes and reduced yields. Moreover, cloning of cDNAs encoding lysine-ketoglutarate reductase or other lysine catabolic enzymes may enable the elimination of this activity in seeds, using such procedures as antisense suppression. Another essential requirement will be to incorporate the increased free lysine and threonine into seed proteins; this may ensure the stable accumulation of both amino acids and minimize negative effects of high levels of these free amino acids on seed development and germination. Incorporation of free lysine and threonine into proteins may be enhanced by transforming plants with genes encoding novel or modified seed proteins that are relatively rich in these amino acids (Wallace et al., 1988).

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