Arrested Embryos from the bio1 Auxotroph of Arabidopsis thaliana Contain Reduced Levels of Biotin

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ABSTRACT

The bio1 auxotroph of Arabidopsis thaliana is a recessive embryonic lethal that forms normal plants in the presence of biotin. The purpose of this study was to determine whether aborted seeds produced by heterozygous plants grown without biotin supplements contained reduced levels of biotin. Two methods were used to determine the biotin content of mutant and wild-type tissues: streptavidin binding in microtiter plates and growth of the biotin-requiring bacterium Lactobacillus plantarum. Total biotin was measured in extracts prepared from immature seeds prior to desiccation. aborted seeds produced by heterozygous (bio1/BIO1) plants contained some biotin in the maternal seed coat but virtually no detectable biotin in the arrested embryo. This lack of biotin was not observed in arrested embryos from other mutants with similar patterns of abnormal development. These results are consistent with the model that bio1 tissues are defective in biotin synthesis. The alternative model of increased degradation is inconsistent with the recessive nature of the mutation and the ability of rescued plants to continue growing for several weeks following removal of supplemental biotin.

The genetic control of plant embryo development has been approached in part through the isolation and characterization of recessive embryo-lethal mutants (20). The most extensive studies have dealt with defective-kernel mutants of maize (31) and embryo-lethal mutants of Arabidopsis (19). The bio1 auxotroph examined in this study was isolated following seed mutagenesis with ethyl methanesulfonate (18) and was chosen for further analysis when arrested embryos were shown to be rescued on media supplemented with amino acids and vitamins (1). Mutant embryos were subsequently shown to produce phenotypically normal plants when cultured in the presence of either biotin or desthiobiotin, the immediate precursor of biotin in bacteria (30). Arrested embryos from other mutants with a wide range of lethal phases did not exhibit this biotin requirement for growth in culture (1, 10). Mutant bio1 plants rescued in culture and subsequently transplanted to soil produced phenotypically normal seeds when supplemented with biotin but became chlorotic and failed to produce fertile flowers in the absence of biotin (30).

Biotin is a heterocyclic cofactor that binds covalently to specific carboxylases to facilitate the transfer of CO2 during carboxylation and decarboxylation reactions (6). The synthesis of biotin in bacteria was elucidated 20 years ago through the analysis of biotin auxotrophs (7). Biotin-dependent carboxylases and biotin operons from various microorganisms have subsequently been studied in detail (8, 27). Several biochemical defects may be responsible for the observed biotin requirement of bio1 arrested embryos and mutant plants. The primary defect could be in biotin synthesis or degradation, the attachment of biotin to carboxylases, the release of protein-bound biotin, intracellular transport of biotin, or a related pathway that regulates biotin synthesis in plants. Several of these defects have been identified among biotin auxotrophs of Escherichia coli (8) and Bacillus subtilis (25) and in human patients with heritable defects in biotin utilization (33). Two types of multiple carboxylase deficiencies have been described in humans (33). The first is caused by a defect in holocarboxylase synthase, the enzyme that attaches biotin to carboxylase apoenzymes. The second is caused by a defect in biotinidase, the enzyme that removes biotin from associated proteins in the diet and generates free biotin for use in growth and development. Both disorders have been shown to respond positively to biotin supplements.

One approach to identifying the biochemical defect in biotin auxotrophs is to measure biotin levels in mutant cells. Defects in biotin synthesis and degradation should reduce the amount of total biotin whereas defects in the attachment and release of biotin from carboxylases should alter only the relative levels of free and bound biotin. We decided to measure the total biotin present in normal and aborted seeds first and then attempt to distinguish between free and bound forms only if mutant tissues contained normal levels of biotin. Two experimental methods were used to assay for total biotin. The first involved coating microtiter plates with streptavidin, a bacterial protein that detects both free and bound forms, and allowing the biotin present in tissue extracts to compete with biotinylated alkaline phosphatase for streptavidin binding sites (2). The second method involved measuring the growth of Lactobacillus plantarum in the presence of plant extracts hydrolyzed with sulfuryc acid to release biotin bound to carboxylases (28).

In this paper we demonstrate that arrested embryos from the bio1 auxotroph of Arabidopsis thaliana contain virtually no detectable biotin. This lack of biotin appears to result from a defect in biotin synthesis that is specific to this mutant and not simply associated with developmental arrest in embryonic lethals. Further analysis of this auxotroph should help to elucidate the biosynthetic pathway for biotin in plants and
the developmental significance of a specific housekeeping function during plant embryo development.

MATERIALS AND METHODS

Plant Material

*Arabidopsis thaliana* (L.) Heyn. strain “Columbia” was grown in pots at 23 ± 3°C beneath 40-W fluorescent lights maintained on daily 16-h light/8-h dark cycles (11). Plants heterozygous for recessive embryo-lethal mutations isolated following EMS^2^ seed mutagenesis (10, 18) were identified by the presence of siliques containing 25% aborted seeds following self-pollination (21). Several mutants with different lethal phases were used as controls for biotin measurements (Table I). Plants in culture were grown on a basal medium containing the inorganic salts of Murashige and Skoog (23), 3% (w/v) glucose, 0.8% (w/v) Difco agar, 0.55 mM inositol, and 5 μM thiamine hydrochloride. Biotin present in enriched media was added prior to autoclaving because its stability at high temperatures is well documented (28). All media were adjusted to pH 5.7 with NaOH, autoclaved for 15 min at 122°C and 18 p.s.i., and poured into 60 × 20-mm sterile Petri plates. Cultures were maintained at room temperature on 16-h light/8-h dark cycles. Rescued *bio1/bio1* seeds germinated in culture were obtained from homozygous mutant plants watered daily with biotin as described previously (30).

Isolation of Seeds and Embryos

Wild-type seeds at various stages of development were isolated from immature siliques under a dissecting microscope and transferred with fine-tipped forceps to 1.5-mL microfuge tubes placed on ice. Developmental stages were determined by seed size and color as well as the position of siliques along the stem. Aborted seeds were collected from heterozygous siliques containing green seeds that had completed morphogenesis but not the final stages of maturation and desiccation. Aborted seeds used for biotin assays were generally inflated and lacked brown pigment in the seed coat. Microfuge tubes containing either 100 or 600 seeds were stored at −20°C until required for preparation of extracts.

Isolated embryos and seed coats were obtained by dissecting normal and aborted seeds on cover slips located on the surface of agar plates lacking biotin. Premature desiccation was prevented by transferring the isolated embryos and seed coats to a small piece of moistened filter paper adjacent to the cover slip. Some of the liquid endosperm was retained along with the seed coats but most was lost during dissection. Filters containing 100 pieces of tissue were transferred to 1.5-mL microfuge tubes and stored at −20°C. The presence of small amounts of filter paper in these preparations did not interfere with biotin measurements.

Preparation of Extracts

Extracts for microbiological assays required acid hydrolysis to release biotin from associated proteins because *Lactobacillus plantarum* responds only to free biotin. Extracts from seeds, embryos, and seed coats were prepared by autoclaving 100 pieces of tissue in 40 μL of 2 N H_2SO_4 for 15 min at 122°C and 18 p.s.i. Further homogenization of acid-hydrolyzed tissue was generally not required. Extracts were neutralized with 16 μL of 5 N NaOH, diluted to 1.5 mL with water, and centrifuged for 1.5 min at 15,600g to remove cellular debris. The resulting supernatant was used immediately for biotin assays. Extracts from seedlings grown in culture were prepared by autoclaving leaf tissue (20–100 mg fresh weight) in 200 μL of acid followed by neutralization with 80 μL of 5 N NaOH, dilution to 1.2 mL with water, and centrifugation. The resulting supernatant was further diluted to 8 mL with water and then used for biotin assays.

Extracts prepared for streptavidin assays were not hydrolyzed with acid because streptavidin binds both free and protein-bound forms of biotin. Groups of 600 seeds were autoclaved in 0.5 mL of PBS for 15 min at 122°C and 18 p.s.i. to facilitate biotin extraction, homogenized briefly with a glass rod, and centrifuged for 1.5 min. The resulting supernatant was used immediately for biotin assays. Leaf and silique extracts were prepared by autoclaving 20 to 100 mg tissue in 1 mL of PBS.

*Lactobacillus* Microbiological Assay

Total biotin in acid-hydrolyzed extracts was assayed with *L. plantarum* (ATCC 8014) by the method of Scheiner (28). Assays were reduced in volume from 10 to 5 mL for seedling extracts and further reduced to 1 mL for seeds, embryos, and seed coats. Standard curves were generated from control tubes containing 0.01 to 0.1 ng/mL biotin. The amount of bacterial growth in each tube was determined by measuring optical density at 600 nm. Dehydrated biotin assay medium was purchased from Difco. All stock solutions, reagents, and sources of deionized water were sterilized to prevent microbial biotin contamination.

Streptavidin Binding Assay

Total biotin in PBS extracts was assayed by modification of the method of Bayer et al. (2). Immulon No. 2 96-well microtiter plates (Dynatech Laboratories) were incubated 3 h at 37°C with 100 μL/well of a 10 μg/mL solution of streptav-

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*Table I. Overview of Mutants Chosen for Biotin Assays*

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Stages of Mutant Embryos</th>
<th>Colors*</th>
<th>Embryos</th>
<th>Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>bio1</em></td>
<td>Globular-mature cotyledon</td>
<td>1–2</td>
<td>2–3</td>
<td></td>
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<tr>
<td>emb20-2</td>
<td>Globular-heart</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>emb29</td>
<td>Globular-linear cotyledon</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>emb24</td>
<td>Globular-mature cotyledon</td>
<td>2–3</td>
<td>2–3</td>
<td></td>
</tr>
<tr>
<td>emb20-3*</td>
<td>Linear-mature cotyledon</td>
<td>1–2</td>
<td>1–2</td>
<td></td>
</tr>
<tr>
<td>111H-2B2</td>
<td>Mature cotyledon</td>
<td>1–2</td>
<td>1–2</td>
<td></td>
</tr>
</tbody>
</table>

* Arrested embryos and aborted seeds were either creamy white (1), very pale yellow-green (2), or pale green (3). * Previously known as *emb26* but recently found to be allelic to *emb20-2.*

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idin in 15 mM sodium carbonate buffer at pH 9.6. Plates were washed four times with PBS, incubated 1 h at 37°C with 100 μL/well of 0.3% (w/v) bovine serum albumin in 15 mM sodium carbonate buffer, and washed again with PBS. Aliquots of 10 ng/mL biotin standard in PBS were added to a series of seven wells in duplicate for generating a standard curve. Standards ranged in concentration from 0.5 to 5.0 ng/mL biotin. Two wells were not incubated with biotin to serve as negative controls. Aliquots of 25 to 100 μL of plant extract were added to the remaining wells in duplicate. The final volume of each well was adjusted to 100 μL with PBS. Plates were incubated 30 min at 25°C with 100 μL/well of a 1 unit/mL solution of biotinylated alkaline phosphatase in PBS. Wells were washed again with PBS, and 100 μL of 1 mg/mL p-nitrophenyl phosphate in substrate buffer (1 M diethanolamine, 100 μg/mL MgCl₂, pH 9.8) was added to each well. Plates were incubated at 37°C for 1 h or until a discernible yellow color appeared in control wells not incubated with biotin standards. Color reactions were stopped by adding 100 μL of 3 N NaOH to each well. The contents of each well were transferred to a 1.5-mL semimicro cuvette and brought to a volume of 1 mL with substrate buffer. Absorbance at 405 nm was measured with a Shimadzu UV-160 spectrophotometer. All reagents for this assay were purchased from Sigma Chemical Co.

RESULTS

Biotin in Plant Tissues

Most cells require only trace amounts of biotin for general metabolism (6). A comparison of biotin levels in mutant and wild-type cells therefore requires sensitive methods of biotin detection. Biotin assays with plant tissues are particularly challenging because so little is known about the biosynthesis and accumulation of biotin in plants (30). Previous studies have been limited to measuring the biotin content of common

<table>
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<th>Table II. Streptavidin and Microbiological Assays of Total Biotin in Mutant and Wild-Type Tissues of A. thaliana</th>
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<tr>
<td>Plant Material Analyzed</td>
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<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Mature green siliques from wild-type plants grown in pots</td>
</tr>
<tr>
<td>Cauline leaves from wild-type plants grown in pots</td>
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<tr>
<td>Rosette leaves from wild-type plants grown on a basal medium</td>
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<tr>
<td>Wild-type seedlings grown for 7–10 d on a basal medium</td>
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<tr>
<td>bio1 seedlings grown for 7–10 d on a basal medium</td>
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<tr>
<td>bio1 seedlings started on 10 nm biotin and then grown for 7 d on a basal medium</td>
</tr>
<tr>
<td>bio1 seedlings started on 5 μM biotin and then grown for 7 d on a basal medium</td>
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</tbody>
</table>

* Each value represents the mean biotin content in pg total biotin per mg fresh weight ± sd.

Table III. Streptavidin and Microbiological Assays of Total Biotin in Mutant and Wild-Type Seeds of A. thaliana

<table>
<thead>
<tr>
<th>Type of Seed Analyzed</th>
<th>pg Biotin per Seed*</th>
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<tbody>
<tr>
<td>Streptavidin Method</td>
<td>Lactobacillus Method</td>
</tr>
<tr>
<td>Wild-type mature cotyledon</td>
<td>3.51 ± 0.88</td>
</tr>
<tr>
<td>Wild-type linear cotyledon</td>
<td>4.26 ± 0.34</td>
</tr>
<tr>
<td>Wild-type globular-heart</td>
<td>1.48 ± 1.11</td>
</tr>
<tr>
<td>bio1 aborted</td>
<td>0.70 ± 0.52</td>
</tr>
<tr>
<td>emb20-2 aborted</td>
<td>2.70 ± 0.00</td>
</tr>
<tr>
<td>emb29 aborted</td>
<td>0.63 ± 0.35</td>
</tr>
<tr>
<td>emb24 aborted</td>
<td>1.40 ± 0.26</td>
</tr>
<tr>
<td>emb20-3 aborted</td>
<td>3.46 ± 1.70</td>
</tr>
<tr>
<td>111H-2B2 aborted</td>
<td>1.17 ± 0.64</td>
</tr>
</tbody>
</table>

* Each value represents the mean of several independent assays ± sd.

feedstuffs (29). The values summarized in Table II were obtained to test the applicability of biotin assay methods to Arabidopsis and to provide a framework for subsequent studies with mutant and wild-type seeds. Biotin levels observed in wild-type tissues of Arabidopsis were within the range of values reported for other plants (29). The reduced biotin content of wild-type plants grown in culture was probably caused by decreased vigor and higher water content of leaves in culture and by the absence of microorganisms that may contribute biotin to plants grown in pots.

We have shown previously that rescued bio1/bio1 seeds germinated on a basal medium turn pale after 7 to 10 d in culture and fail to develop beyond the cotyledon stage (30). Results summarized in Table II are consistent with the model that these homozygous mutant seedlings stop growing when they deplete residual biotin obtained from parental plants. Senescence can be delayed and biotin levels increased by germinating rescued seeds on plates containing biotin and then transferring mutant seedlings to a basal medium. The loss of biotin in mutant plants is therefore not simply an indirect consequence of senescence.

Biotin in Intact Seeds

The remainder of this study dealt with biotin levels in seeds produced by plants grown in the absence of supplemental biotin. Results of biotin assays with mutant and wild-type seeds are summarized in Table III. Biotin contents were calculated as pg/seed rather than pg/mg fresh weight because the precise weight of small groups of seeds could not be readily determined. If we assume an average weight of 30 μg/seed as reported previously (11), the amount of biotin detected in wild-type seeds at the cotyledon stage of development (3–4 pg/seed or approximately 100 pg/mg fresh weight) was equivalent to the value reported in Table II for intact siliques. Lower levels of biotin were found in wild-type seeds at earlier stages of development.

Results obtained with the streptavidin and microbiological assays were remarkably consistent considering the small amounts of tissue being analyzed. One difference between
these methods was that streptavidin bound to both desthiobiotin and biotin whereas *Lactobacillus* responded only to biotin. Streptavidin assays therefore measured combined levels of biotin and desthiobiotin in tissue extracts. The similarity of results obtained with these methods as shown in Table III demonstrates that desthiobiotin does not accumulate to high levels in plant tissues.

Biotin levels in aborted seeds from six mutant lines with characteristic lethal phases and patterns of abnormal development (Table I) were determined using both the microbiological and streptavidin methods (Table III). Aborted seeds from every mutant line tested contained at least some biotin prior to desiccation. Biotin deficiency is therefore not a common feature of developmental arrest in embryo-lethal mutants. Aborted seeds produced by *bio1* heterozygotes appeared to contain lower levels of biotin than wild-type seeds at an equivalent (linear) stage of development, but the differences were not particularly striking, and at least one other mutant (emb29) produced aborted seeds that appeared with streptavidin assays to contain depressed levels of biotin. The position of heterozygous siliques along the stem and differences in the developmental stages of arrested embryos may also have contributed to some of the observed variability in biotin content of mutant seeds. Assays with intact seeds could therefore not provide a definitive answer to the question of whether *bio1* tissues contained unusually low levels of biotin.

**Biotin in Isolated Embryos**

Aborted seeds produced by phenotypically normal *bio1/BIO1* plants were expected to contain at least some biotin in the maternal seed coat. This heterozygous tissue constitutes a significant portion of the fresh weight of immature seeds and should not have been defective in either biotin synthesis or transport. The question then became whether the biotin detected in *bio1* aborted seeds was located exclusively in the seed coat or whether part of this biotin was present in the arrested embryo. We chose to address this question by performing biotin assays with groups of isolated embryos and seed coats obtained from the same collection of aborted seeds. The results of these experiments as summarized in Table IV demonstrated conclusively that *bio1* aborted embryos contained only trace amounts of biotin and that all of the biotin detected in *bio1* aborted seeds could be attributed to the maternal seed coat.

The absence of biotin in *bio1* aborted embryos could not be explained by either the small size or pale color of mutant embryos. The five mutants chosen as controls for these experiments produced arrested embryos that were either slightly smaller (emb20-2, emb29), equivalent (emb24), or slightly larger (emb20-3, 111H-2B2) than *bio1* aborted embryos. Arrested embryos from emb20-2 and emb29, which were on the average less than half the size of *bio1* aborted embryos, contained almost 10 times more biotin than *bio1* aborted embryos. Both of these mutants also produced arrested embryos that were paler than *bio1* aborted embryos. The depletion of biotin in *bio1* aborted embryos was therefore not an indirect consequence of reduced Chl accumulation or chloroplast function. Biotin levels in isolated embryos and seed coats (Table IV) even correlated reasonably well with values obtained for intact seeds (Table III). The absence of biotin in *bio1* aborted embryos was therefore not caused by the loss of liquid endosperm during dissection. All of these results are consistent with the model that *bio1* aborted embryos lack biotin because they are defective in biotin synthesis.

**DISCUSSION**

Auxotrophic mutants with defined nutritional requirements have played an important role in the genetic dissection of biochemical pathways in microorganisms. Many of the gene identified by these mutants have been examined in detail at the molecular level. The scarcity of plant auxotrophs has greatly limited the application of a similar approach to the analysis of plant metabolism (3, 16). As a consequence, many of the genes required for the biosynthesis and utilization of amino acids and vitamins in plant cells remain to be identified. Several models have been proposed to explain the scarcity of plant auxotrophs, including the presence of gene duplication, alternative biochemical pathways, coupling of biochemical and photosynthetic processes, limited transport of essential nutrients, and lethalality during gametogenesis or embryogenesis (3, 14). Most of the existing plant auxotrophs have been isolated through selection of variant cell lines (3) and mutant seedlings (15) in culture and the identification of mutant embryos (1, 32) and plants (17, 26) that respond to nutritional supplements. The most extensive studies have dealt with thiamine (13), biotin (30), and tryptophan (15) mutants of *Arabidopsis*.

The biotin auxotroph of *Arabidopsis* examined in this study represents the first example of an embryonic lethal with a defined biochemical defect early in embryogenesis that can be rescued by the addition of a specific nutrient. Further analysis of this mutant should provide important clues to the nature of plant auxotrophs, the biosynthesis and utilization of biotin in plants, and the underlying causes of abnormal development in embryo-lethal mutants. Arrested embryos from lethal mutants of *Arabidopsis* have been shown previously to differ with respect to size and color, stage of developmental arrest, response in culture, ultrastructure, accumu-

| Table IV. *Lactobacillus* Microbiological Assay of Total Biotin in Embryos and Seed Coats Isolated from Mutant and Wild-Type Seeds of *A. thaliana* |
|---------------------------------|-----------------|-----------------|
| Type of Seed Analyzed           | pg Biotin per Individual* |                 |
|                                 | Embryo          | Seed Coat       |
| Wild-type linear cotyledon      | 0.39 ± 0.06     | 0.59 ± 0.25     |
| *bio1* aborted                  | 0.03 ± 0.04<sup>b</sup> | 0.72 ± 0.24 |
| *emb20-2* aborted               | 0.26 ± 0.16<sup>c</sup> | 0.67 ± 0.14 |
| *emb29* aborted                 | 0.22 ± 0.13<sup>c</sup> | 0.96 ± 0.40 |
| *emb24* aborted                 | 0.31 ± 0.10<sup>c</sup> | 0.93 ± 0.16 |
| *emb20-3* aborted               | 1.65 ± 0.82<sup>c</sup> | 1.04 ± 0.50 |

* Each value represents the mean of several independent assays ± s.d.<sup>*</sup>
<sup>b</sup> Significantly lower than wild-type and mutant controls at P < 0.05 determined by t-test.
<sup>c</sup> Not significantly different from wild-type control at P < 0.05 determined by t-test.
lation of storage proteins, and patterns of gametophytic gene expression (19, 20, 22). Many of these mutants are likely to be defective in housekeeping functions that first become critical during embryogenesis. Other mutants may help to identify genes with important regulatory functions specific to embryogenesis. Both types of embryonic lethals should facilitate the genetic analysis of plant development.

Very little is known about the biosynthesis and utilization of biotin in plants (34). The site of biotin synthesis, the identity of early biochemical intermediates, and the nature of biotin transport remain to be determined. The most thoroughly studied biotin-dependent enzyme in higher plants is acetyl-CoA carboxylase, which performs an essential function during the initial stages of fatty acid biosynthesis (5, 24). Several other carboxylases that require biotin for normal function have also been identified (12, 36). Cell lines with elevated levels of biotin have been recovered following selection for resistance to pimelic acid (35) but these variants were not regenrated into plants or examined in detail. Most of the information on biotin synthesis and transport in higher plants has come from analysis of the biotin auxotroph described in this report. We have shown previously that biotin can be transported from the soil to immature seeds where it can rescue biol/biol embryos produced by heterozygous and homozygous mutant plants (30). Maternal sources of biotin are insufficient to rescue mutant embryos produced by heterozygous plants grown in the absence of supplemental biotin. We have also shown that bio1 arrested embryos and mutant seedlings are rescued when grown in the presence of either desthiobiotin or diaminopelargonic acid, the final intermediates of biotin synthesis in microorganisms (8). At least the final stages of biotin synthesis in bacteria are therefore conserved in higher plants. The present study has extended our analysis of this biotin auxotroph by demonstrating that arrested embryos are indeed deficient in biotin.

Auxotrophs may be defective in either the biosynthesis, degradation, transport, or utilization of an essential nutrient. The results presented in this report suggest that bio1 arrested embryos are defective in either the biosynthesis or degradation of biotin. Several lines of evidence are potentially inconsistent with increased degradation. Mutant embryos are rescued by extremely low levels of biotin in culture and continue to grow for several months following subculture to fresh media lacking biotin (30). Mutants with high rates of biotin turnover would be expected to deplete this supplemental biotin more rapidly. The ability of mutant embryos to be rescued by low levels of desthiobiotin is particularly difficult to explain with this model if mutant cells degrade biotin as quickly as it becomes available. Increased degradation also represents a gain of function that should be inherited as a dominant trait, in contrast to a biosynthetic defect, which represents a loss of function more likely to be inherited as a recessive trait. We therefore believe that bio1 embryos are defective in biotin synthesis, and that the block occurs prior to the conversion of diaminopelargonic acid to desthiobiotin.

Further analysis of this biotin auxotroph continues to be limited by the absence of a reliable source of 7-keto-8-amino-pelargonic acid, the precursor of diaminopelargonic acid in bacteria. We are therefore continuing efforts to purify this compound (9) from supernatants of the corresponding bioA auxotroph of E. coli (7). We have also mapped this mutation to a position within 3 cM of the tz locus on chromosome 5 and have begun to test RFLP probes in this region (4) in preparation for gene isolation through chromosome walking. We have also initiated an attempt to rescue mutant cells with the bacterial bioA and bioF genes introduced through Agrobacterium-mediated plant transformation. These biochemical and molecular studies should help to elucidate the early steps of biotin synthesis in plants and the specific role of the B01 gene product in plant growth and development.

ACKNOWLEDGMENTS

We thank Ken Robinson for maintaining plants and Leigh Mickelson for assistance with seed dissections.

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