Coordinated Transcriptional Regulation of Storage Product Genes in the Maize Endosperm

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We have demonstrated that expression of genes involved in starch and storage protein synthesis of the maize (Zea mays L.) endosperm are coordinated. Genetic lesions altering synthetic events in one biosynthetic pathway affect expression of genes in both pathways. Initial studies focused on shrunken2 (sh2) and brittle2 (bt2) mutants because these genes encode subunits of the same enzyme, ADP-glucose pyrophosphorylase. Analysis of various sh2- and bt2-mutant alleles showed that the most severe mutations also conditioned the largest increase in transcripts. The analysis was extended by monitoring the transcripts of the genes, shrunken1 (sh1, structural gene for Suc synthase), sh2, bt2, waxy1 (wx1, structural gene for starch synthase), and those of the large and small zeins in isogenic maize lines at 14, 22, and 30 d postpollination. Enzymes were wild type for all of these genes or contained sh1-, sh2-, bt1-, bt2-, opaque2 (o2-), or amylase-extender1 (ae1-) dul1 (du1-) wx1- mutations. Transcripts increased continually throughout kernel development in the mutants relative to the standard W64A used. Variation in the amount of Suc entering the developing seed also altered transcript amounts. The results indicate that starch and protein biosynthetic genes act in a concerted manner, and both are sensitive to mutationally induced differences.

The maize (Zea mays L.) endosperm is a specialized tissue that provides a source of nutrients to the germinated embryo. The vast majority of carbon used in the early steps of seedling development is derived from starch and the storage proteins, the zeins. These storage products account for approximately 90% of the dry weight of a mature maize endosperm.

Historically, mutants have provided the most insight into the pathways of starch and zein synthesis. Many endosperm mutations can be classified as affecting starch or zein biosynthesis. Mutations such as o2- (=floury2-), and o7- decrease zein content, and proteins in the other solubility classes increase in abundance (Mertz et al., 1964; Nelson et al., 1965; Ma and Nelson, 1978; Habben et al., 1993). o2 has been shown to encode a trans-acting regulatory protein involved in zein transcription (Schmidt et al., 1990). S and L zeins represent the 19- and 22-kD classes of zein polypeptides and an o2-mutation affects mainly the production of 22-kD zein proteins (Langridge et al., 1982).

The majority of the enzymes known to be involved in the starch biosynthetic pathway follow similar developmental timing (Tsai et al., 1970). Ingle et al. (1965) found maximum endosperm sugar content at 22 dpp. This stage of development is also when the starch biosynthetic enzymes are most active (Tsai et al., 1970). Mutations in the genes sh1 (the structural gene for Suc synthase; Chourey and Nelson, 1976), bt1 (the structural gene for a membrane-bound, adenylyl carrier; Sullivan et al., 1991), and sh2 and bt2 (both structural genes for AGP; Hannah and Nelson, 1976) decrease starch content in the mature kernel as a consequence of the loss of an important starch synthetic enzyme or protein. Mutations in wx1 (the structural gene for the starch-bound ADP-Glc glucosyl transferase; Nelson and Rines, 1962), ae1 (the structural gene for a starch-branching enzyme; Boyer and Preiss, 1978), and du1 (Creech, 1965) do not reduce starch amount but rather alter the ratio of the two major starch constituents, amylose and amylopectin. The straight-chained amylose is abolished in wx1- mutations, whereas ae1- and du1- mutations increase the amount of amylose relative to the amount of amylopectin. Neither ae1- nor du1- reduces the total amount of starch by more than 20% (Creech, 1965). The triple mutant ae1- du1- wx1- results in kernels with increased sugars and reduced starch similar to sh2-, bt2-, and bt1-mutants (Creech, 1965). More detailed descriptions of these mutants can be found in a recent review (Hannah et al., 1993).

Although mutations are historically placed into the starch or zein biosynthetic pathways, hints of possible interactions between these pathways have been noted. For example, Tsai et al. (1978) and Barbosa and Glover (1978) reported that the sh2-, bt2-, and bt1- mutations that severely reduce starch synthesis also reduce zein synthesis to the same extent as o2-.

Ample evidence exists that expression of various genes is modulated by sugar concentration. For example, studies with maize protoplasts revealed repression of transcription of photosynthetic genes by various carbon sources (Sheen, 1990).

Abbreviations: ae1, amylase-extender1; AGP, ADP-Glc pyrophosphorylase; agp1, ADP-Glc pyrophosphorylase1; agp2, ADP-Glc pyrophosphorylase2; bt1, brittle1; bt2, brittle2; dpp, days postpollination; du1, dul1; L zein, large zein; o2, opaque2; S zein, small zein; sh1, shrunken1; sh2, shrunken2; sus1, Suc synthase1; su1, sugary1; wx1, waxy1.

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In potato, expression of one of two starch AGP genes was increased in response to elevated Suc concentrations (Muller-Rober et al., 1990). With regard specifically to maize starch biosynthetic genes, Koch et al. (1992) showed that expression of wild-type alleles of sh1 and sus1, the genes encoding the two forms of Suc synthase, was modulated in maize roots by sugar content. The amount of Suc leading to maximal sh1 expression was less than that for sus1. These observations, taken together, suggest that the response of these genes relates to their role in tissues serving as a source or sink for carbohydrates. Accordingly, the maize endosperm might be expected to respond to high sugar concentrations by increasing the amount of transcripts present. In previous studies (Bae et al., 1990), we noted that mutation at either of the two structural genes (sh2 and bt2) for AGP resulted in increased steady-state levels of transcripts of the other nonallelic and nonmutant structural gene. Further studies were designed to focus on the coordination of expression between these two genes as detected by mutational analysis. It was discovered that this networking in expression is more global than just these two genes, reflects the severity of the genetic block, and involves the expression of genes important for at least starch and protein synthesis. The mutant effect can be mimicked in wild-type seed by increases in the sugars presented.

**MATERIALS AND METHODS**

**Nomenclature**

Names and symbols for genes, their alleles, and their products follow the 1993 version of maize genetics nomenclature (Beavis et al., 1993). When reference is made to the gene or to its alleles, the abbreviation is italicized; for example, sh2 is used to refer to this locus. Alleles beginning with a capital letter, e.g. Sh2-, refer to the functional or dominant form of the allele, whereas recessive alleles are designated with a lowercase first letter, e.g. sh2-. A hyphen follows the allelic symbol and the designation of the particular allele, e.g. sh2-R, is given when known or relevant. Gene products are not italicized. Proteins are given in all capital letters, e.g. SH2. Transcripts, as suggested by Oliver Nelson, chairman of the Maize Nomenclature Committee, are distinguished by the nonitalicized gene symbol; the first letter is capitalized when the transcript arises from a functional wild-type allele.

**Plant Culture**

The sh2- and bt2- mutant alleles were described previously (Hannah and Nelson, 1975, 1976). The W64A isogenic lines were wild type or contained the mutant alleles, sh1-, sh2-, bt1-, bt2-, ao2-, ae1-, du1-, or wx1- as well as ae1- du1- wx1-. The W64A isolines were grown in a greenhouse with temperatures of 33°C (day) and 24°C (night). Developing ears were utilized as a source of kernels for RNA, protein, and run-on transcription analysis.

**RNA Isolation**

RNA was isolated from kernels that had been quick-frozen in liquid N2. The samples were ground to a fine powder with a mortar and pestle, and RNA was isolated by the LiCl method of McCarty (1986). Total RNA was utilized in the northern blot analysis.

**RNA Gel Blots**

RNA samples were denatured in 2.2 M formaldehyde/50% formamide and fractionated on 1.5% agarose/2 M formaldehyde gels using a Mops buffer (Maniatis et al., 1982). RNA gels were blotted onto nylon membrane (Hybond N, Amsham) following standard protocols (Maniatis et al., 1982). The transfer buffer was 10X SSPE (1.8 M NaCl, 100 mM Na2HPO4 [pH 7.7], and 10 mM EDTA). After blotting, samples were cross-linked to the wet nylon membrane by 6 min of UV irradiation (3 J/cm²).

Hybridizations were in 0.5 M Na2HPO4 (pH 7.2), 7% SDS, and 1% BSA (Church and Gilbert, 1984). Blots were prehybridized for 1 h at 65 to 67°C, and hybridizations were for 16 to 20 h, also at 65 to 67°C. Probes were prepared by the random primer method (BRL protocols) to a specific activity of greater than 5 x 10⁶ cpm/μg cDNA. Blots were washed twice in 2X SSPE, 0.1% SDS and twice with 0.1X SSPE, 0.1% SDS. Each wash was for approximately 30 min at 65 to 67°C. Filters were dried and subjected to autoradiography. Signals were quantified using an Ambis radioanalytical imaging system. Filters were reprobed with an rDNA clone, and data were adjusted for minor loading discrepancies. Data in Tables I, II, and III using replicated RNA or nuclear samples from kernels of greenhouse-grown maize gave values that were within ±25%.

**Protein Analysis**

Tissue for protein samples was ground to a fine powder with a mortar and pestle in liquid N2 and added to an equal weight:volume of buffer containing 0.1 M Tris-HCl (pH 7.8), 10 mM DTT, and 20% glycerol. 2-Mercaptoethanol was added to 5%, and the samples were heated to 90°C for 3 min. Protein determinations were as described by Bradford (1976) using a BSA standard. SDS-PAGE was by the method of Laemmli (1970). Proteins were transferred to nitrocellulose membranes (BA-85 0.45 μm, Schleicher & Schull) by standard protocols (Ausubel et al., 1987). Ponceau S staining provided visual confirmation of even protein loading and electroblot transfer (Ausubel et al., 1987).

Immunoblotting were performed according to protocols supplied by Bio-Rad using 1% BSA to block nonspecific antibody binding. A 1:300 dilution of SH2, BT2, or preimmune antiserum in Tris-buffered saline with 1% BSA was used. The preparation of the SH2 and BT2 antibodies was described previously (Giroux and Hannah, 1994). Antibody binding was recognized by binding of the secondary antibody, goat anti-rabbit (Bio-Rad), linked to an alkaline phosphatase conjugate and developed with the color reaction utilizing 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium (BRL; Ausubel et al., 1987).

SH2 and BT2 protein levels were quantified using enhanced chemiluminescence protocols and materials (Amer sham). Development of the blots utilized the binding of the secondary antibody, goat anti-rabbit (Bio-Rad), linked to a
horseradish peroxidase activity. Several exposures of each blot were developed, and bands corresponding to SH2 and BT2 protein subunits were quantified using a scanning densitometer (Molecular Dynamics, Sunnyvale, CA).

AGP Activity

The determination of AGP activity was performed as described by Hannah and Nelson (1975). Activity values presented in Table I represent amounts relative to the standard in the presence of 20 mM 3-phosphoglyceric acid.

Isolation of Nuclei and Run-On Analysis

Nuclei were obtained from kernels that had been quick-frozen in liquid N₂. The nuclei were isolated, and the run-on transcription assays were performed according to the method of Walling et al. (1986). Quantification of nuclei was by UV spectroscopy. Labeled transcripts were isolated according to the method of Manley et al. (1983). Addition of 2 μg/mL 3-amaminin decreased incorporation below detection. Hybridizations were with 7.5 μg of each plasmid on Southern blots using hybridization and washing conditions for the DNA and RNA blots. Blots were quantified with a Molecular Dynamics Phosphorimager. Values were standardized to the rDNA signals.

In Vitro Kernel Culture

Blocks of W64A × 182E F₂ kernels were isolated at 22 dpp from the field-grown maize and surface sterilized according to the methods of Gengenbach (1977). They were placed on filter paper in Petri plates moistened with filter-sterilized Suc or mannitol solutions. Plates were incubated for 4 d at 22°C in the dark. Kernels were quick-frozen, RNA was isolated, and the transcript amounts were determined as described above.

RESULTS AND DISCUSSION

Expression of Starch Synthetic Genes in Selected Starch Mutants

Previously we reported that the Sh2 transcript was elevated in bt2- mutants and the Bt2 transcript was elevated in sh2- mutants (Bae et al., 1990). Since Sh2 and Bt2 encode subunits of one enzyme, AGP, and because the genes likely share a common evolutionary origin (Bae et al., 1990; Bhave et al., 1990), we entertained the idea that the data from the mutants were uncovering a form of end-product feedback control. Alternatively, the elevated transcripts could reflect a more global effect, perhaps related to changes in amounts of a metabolite involved in starch synthesis. To determine whether the elevation of Sh2 and Bt2 transcripts was confined to sh2- and bt2- mutants, we assayed transcript and protein amounts in additional mutants that affect starch content (Fig. 1).

In agreement with previous data, the relative amount of Sh2 transcript is increased in bt2- mutants and the Bt2 transcript is increased in sh2- mutants (Fig. 1A). That the increase is not confined to AGP structural genes is also shown in Figure 1. The relative amount of Sh2, Bt2, and Sh1 transcripts present in sh2-, sh1-, bt1-, and bt2- mutants at 22 dpp was analyzed by the quantification of northern blots. The largest increases in Bt2 transcript were in the sh2- and bt1- mutants, whereas no increase in sh1- was observed. Likewise, the Sh2 transcript was increased in both the bt2- and bt1- mutants, but in sh1- it was relatively unaffected. There was a noticeable but small increase in the amount of the Sh1 transcript in bt1- and bt2- mutants. Since both the Sh2 and Bt2 transcripts were increased in the bt1- mutant and Bt1 is the structural gene for an adenylate carrier (Sullivan et al., 1991), we conclude that the signal causing the increased Sh2 and Bt2 transcripts is not the loss of these transcripts and functional AGP activity but rather is related to some other change in the starch biosynthetic pathway.

Additionally, we asked whether the increased transcript content led to an increased amount of protein. The use of SH2- and BT2-specific antibodies showed that SH2 protein content led to an increased amount of protein. The use of SH2- and BT2-specific antibodies showed that SH2 protein content led to an increased amount of protein.
increased in both the \(btl^1\) and \(bt2^2\) mutants (Fig. 1B), and the BT2 protein increased in \(sh2^1\) and \(bt1^1\) mutants (data not shown). These data point to \(Sh2\) and \(Bt2\) transcript content as one (and perhaps the major) limiting step in the synthesis of the two AGP subunits and are consistent with the observed increase in enzymic activity associated with an increase in the number of functional \(Sh2^1\)- and \(Bt2^2\)-alleles (Hannah and Nelson, 1975).

The \(Sh2\) and \(Bt2\) transcripts barely increased in the \(sh1\)-mutant. The product of \(sh1\), Suc synthase, comes early in the starch biosynthetic pathway, whereas the proteins of the \(sh2\), \(bt2\), and \(bt1\) genes function much later. Possibly, elevation in a metabolite following cleavage of Suc, but before the synthesis of ADP-Glc, is involved in the response. However, \(sh1\)-mutants differ from most \(sh2\)-, \(bt2\)-, and \(bt1\)-mutants in that the latter are associated with a more severe block in the pathway, resulting in lower starch content and greater increases in soluble sugars. Conceivably, then, the differences noted in Figure 1 reflect differences in the severity of the genetic block.

To address this question, we examined two \(sh2\)-mutant alleles of varying severity. Although most \(sh2\) mutants are phenotypically indistinguishable, the mutant allele \(sh2^1\), isolated by M. Neuffer, leads to a phenotype intermediate between wild type and the standard \(Sh2\)-allels used. To determine whether elevated transcripts are in kernel components other than the endosperm, transcripts in \(sh2^2-R\) and \(sh2^1\)-embryos and endosperms were determined (Table II). Although small increases were noted in embryo transcripts, much larger increases in transcripts were noted in the endosperm. This was expected since the genes examined are endosperm specific and are involved in the production of endosperm storage compounds.

The source of the transcript increase in \(sh2^1\), \(sh2^2-R\), and \(bt2^2-B\) was addressed with run-on transcription assays (Table III). Transcription rates of endosperm-specific starch biosynthetic genes were elevated and were comparable to the increases in the steady-state levels of transcripts. The increased \(sh2^1\)-transcript relative to standard \(W64A\times 182E\) \(F_2\) kernels likely represents part of the response of starch genes to feedback control. Transcription of \(sh2^2-R\) was undetectable. \(sh2^2-R\) has an insertion in the \(5^\prime\) region of the transcribed region (J.R. Shaw, M.J. Giroux, and L.C. Hannah, unpublished data). We conclude that the elevation in the nonmutant transcripts is a consequence of increased transcription rather than an effect at the posttranscriptional level.

### Transcript Increases Exist in Additional Starch Biosynthetic Mutants

To extend these analyses to other mutations affecting storage products of the maize endosperm, inosogenic lines containing a number of endosperm-specific mutations were examined. The \(W64A\) inosogenic lines utilized were wild type or contained a single \(sh1^1\), \(sh2^1\), \(bt1^1\), \(bt2^2\), or \(oa^2\)-mutation. A \(W64A\) isolate containing the triple mutant \(oa^1\)-\(du1\)-\(wx1\)-was also used. Table IV presents \(Sh1\), \(Sh2\), \(Bt2\), \(Wx1\), \(S\) zein, and \(L\) zein transcript amounts at early (14 dpp), middle (22 dpp), and late (30 dpp) stages of development. Representative northern blots of 22-dpp kernels are shown in Figure 2. The middle stage (22 dpp) is recognized as the peak of starch synthesis.

### Table II. \(Bt2\), \(S\) zein, \(Agp2\), and \(Wx1\) transcript content of the same \(sh2\)- and \(bt2\)-mutants shown in Table I and embryo (Emb)- and endosperm (Endo)-specific transcripts in selected \(sh2\)-mutants at 22 dpp

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<tr>
<th>Transcript</th>
<th>Relative Steady-State Transcript Amount</th>
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<td>(Bt2)</td>
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<td>(S) zein</td>
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<td>(Agp2)</td>
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<td>(Wx)</td>
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The transcripts were measured from northern blots containing 7.5 \(\mu\)g of total RNA at 22 dpp and expressed relative to our standard wild type.
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Table III. Transcription rates of endosperm-specific genes in selected sh2- and bt2- mutants

Nuclei were extracted from intact kernels at 22 dpp. Transcription rates were expressed relative to standard W64A × 182E F2. Data are the averages of two or more experiments, so values ranged from 1 to 23%.

<table>
<thead>
<tr>
<th>Transcript</th>
<th>Relative Transcription Rates</th>
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<td>Sh1</td>
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<td>Wx1</td>
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<td>S zein</td>
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biosynthesis (Ingle et al., 1965; Tsai et al., 1970). Mutant transcript content is expressed as a proportion of that in the standard W64A kernels in which transcripts of the starch synthetic genes, per total RNA, are highest at 14 dpp, remain high, and then rapidly decline at approximately 25 dpp (data not shown). Amounts at 30 dpp represent only 5 to 15% of those seen at 14 dpp.

If the data are expressed on a protein or endosperm basis, a peak at 22 dpp occurred with most transcripts. Furthermore, AGP and Sh2 and Bt2 transcript developmental profiles were virtually superimposable in wild type when the data were expressed on a kernel or protein basis (data not shown). The decline in Sh2 and Bt2 transcripts occurred more rapidly than that reported recently by Prioul et al. (1994); however, this most likely represents differences in growing conditions.

At 14 dpp, the greatest increases in the endosperm-specific transcripts were in bt1-, bt2-, and ae1- du1- wx1- mutants (Table IV). These mutants severely block the starch biosynthetic pathway. The Bt2 and Wx1 transcripts were the most highly elevated at this stage of development. The increases relative to wild type were a maximum of 478% in the sh2- mutant for the Bt2 transcript. Sh1, S zein, and L zein transcripts decreased or were only moderately increased.

The increases at 14 dpp were accentuated at 22 dpp (Table IV). The greatest increases (more than 5-fold) were again observed in the mutants in which starch synthesis is severely blocked. At 30 dpp, this pattern was amplified (Table IV). Increases of 10-fold or more occurred in the sh2-, bt1-, bt2-, ao2-, and the ae1- du1- wx1- mutants. The Sh2 and Wx1 transcripts were most affected.

A surprising observation was the increased accumulation of the starch synthetic transcripts in the o2- mutant. Increases of approximately 10-fold were noted in the Sh1 and Sh2 transcripts at 30 dpp. Since O2 is involved in zein biosynthesis with no reported effects on starch biosynthesis, this result was unexpected. Since starch synthesis transcripts were altered in the o2- mutant, we asked whether zein transcripts might be altered in the starch mutants.

At 14 dpp, there were small increases in S and L zein transcripts in sh2- (Table IV). Decreases in these transcripts relative to the standard W64A were observed in the remainder of the mutants. At 22 dpp, there were increases in the zein transcripts in most starch mutants (Table IV). The greatest increases were in bt1- and in bt2-. Greater than 10-fold increases occurred in sh1-, wx1-, and ae1- du1- wx1- at 30 dpp in S and L zein transcripts (Table IV). The lowest overall increases were in bt1- and o2-. Large increases in S zein transcript were not unexpected since O2 affects mainly the production of 22-kD (L) zein proteins.

The expression of genes not known to affect the production of storage products in the maize endosperm were also followed in the mutants. Clones of the genes, agp1 and agp2, embryo counterparts of sh2 and bt2, respectively (Girolou and Hannah, 1994), and sus1, which encodes Suc synthase primarily in the embryo (McCarty et al., 1986), were used to compare transcripts in 22- and 30-dpp kernels (Table IV). Although there were some increases in these transcripts at 22 dpp (e.g. a 4-fold increase in Agpl transcript in ae1- du1- wx1-), the increase in transcripts conditioned by these mutants was generally less than that observed for endosperm-specific storage product genes. However, increases in the embryo genes would be expected if they were induced by mechanisms that affect the kernel as a whole and that were not simply endosperm specific. Although the sh2 and bt2 genes are endosperm specific and do not reduce the AGP activity in the embryo, sugar concentrations are elevated in mutant embryos. TB-A translocations were used to generate seed having a wild-type embryo but a sh2- mutant endosperm. Embryos of such seed had an elevated sugar content (C. Parera, D. Cantliffe, D.R. McCarty, and L.C. Hannah, unpublished data).

SH2 and BT2 Proteins Are Not Elevated to the Same Extent as Their Transcripts in the Starch Mutants

Because of the magnitude of the increase in transcripts noted in the mutants conditioning severe blocks in starch synthesis at 30 dpp, we asked whether the cognate protein was also increased to the same extent. SH2 and BT2 proteins were measured in the isogenic mutants at 14 and 30 dpp (Fig. 3). Although the proteins were elevated in some of the severely blocked starch mutants (bt1- and the triple mutant ae1- du1- wx1-) as well as wx1- and du1-, the increase was not nearly as great as noted for the transcripts. Mutants not increasing transcripts showed no increase in proteins. The approximate 2-fold increase in the amount of SH2 and BT2 protein in bt1- is in agreement with the reported elevated AGP activity in developing bt1- kernels (Doehlert and Kuo, 1990).

Interestingly, both BT2 and SH2 proteins were reduced in

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Transcription Increases Coincide with Increased Transcripts

Nuclei isolated from standard W64A, bt1-, bt2-, and wx1-mutant kernels at 30 dpp were used in nuclear run-on assays, and transcription of the Sh1, Sh2, Bt2, Wx1, and S zein genes were monitored. Figure 4 presents transcriptional activity in the mutants relative to the standard W64A. The increases are of the same magnitude as the increased steady-state transcript content. Increased transcription rates apparently account for the increase in steady-state transcript, as has been shown for hordein B and C genes (Sorenson et al., 1989).

Influence of Suc and Mannitol on Transcript Amounts

The increased transcripts in the starch-deficient mutants could be due to a buildup in a metabolite(s) in the synthesis of starch or to lower starch content. In an attempt to distinguish between these possibilities, an in vitro kernel development procedure was used to alter the amount of Suc entering wild-type seed. If the response in the mutants is due to optimal concentrations. Conversely, if the mutant response is due to elevated sugar concentrations, a positive correlation between transcript amounts and sugars would be expected.

Cobb and Hannah (1983) reported that maize kernels grown in vitro develop normally, resemble field-grown kernels, and develop to maturity at 30 to 35 dpp when supplied with relatively high Suc. In subsequent studies, Cobb et al. (1988) showed that lower Suc concentrations (0.06–0.3 M) led to suboptimal growth, as judged by lowered kernel weight.

Table V shows the resulting transcript content when seeds...
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Figure 2. Representative northern blots of isogeneic developing kernels in a W64A background harvested at 22 dpp. These blots are representative of the data presented in Table IV. Area shown is the region of hybridization for each of the listed transcripts.

Figure 3. Western analysis of the SH2 and BT2 proteins in isogeneic lines at 14 and 30 dpp. The relative protein content of SH2 and BT2 was determined in the W64A genotypes as listed for each figure. Total buffer-soluble protein (20 µg) was loaded for each genotype in A, B, C, and D. The SH2 antibody was used in A and B and the BT2 antibody was used in C and D. A and C represent protein samples from 14-dpp kernels and B and D represent protein samples from 30-dpp kernels. Data are the average of two experiments and are expressed relative to the standard W64A (WT). SD values ranged from 0 to 21%.

Figure 4. Relative run-on transcription at 30 dpp in isogeneic nuclei. A and B are Southern blots with lanes containing Sh1, SH2, BT2, Wx1, and S zein cDNAs. Blot A was probed with labeled transcripts from wild-type nuclei, and blot B was probed with transcripts from bt2− nuclei. Blots probed with RNA from bt1− and wx1− are not shown. An rDNA clone was used as a control for the total amount of hybridization and as a marker for correct film exposures. The counted values from a Molecular Dynamics Phosphorimager were corrected based on the rDNA value for each blot, and final corrected transcription rate values are presented in C. Data presented in C are averages of two or more experiments. so values ranged from 10 to 28%.

developed on varying concentrations of Suc or mannitol. SH2 and BT2 transcripts increased as the Suc in the growth medium was elevated, whereas the Sh1 and S zein transcripts were much less responsive. This differential response of the transcripts to alterations in Suc in the growth medium mimics the alterations in transcripts of the high sugar/low starch mutants (sh2−, bt2−, bt1−, and the triple mutant ae1− du1− wx1−). Furthermore, since the low concentrations of Suc in the growth medium give rise to reduced seed weight (Cobb et al., 1988) and presumably lowered starch content, the signal leading to the elevation in transcripts may involve an increase in sugars rather than the reduced starch content. The transcripts most increased, SH2, BT2, and Wx, were also those most involved in the type and amount of starch produced. This argues that genes directly involved in producing storage products in the endosperm are most sensitive to an increase in metabolically active sugars. SH2 and BT2 transcripts were also modulated by varying mannitol in the growth medium. Although the pattern of change was less pronounced than that seen with Suc, the alteration in transcript amounts may be a response to altered osmotic conditions rather than a direct effect of Suc.
showed that Shl and Sus1 expression in maize root tips is a distinctive profile. Taken together, the evidence indicates maize protoplasts relative to a mannitol control (Sheen, 1990) would be repressed by Suc and those involved in the production of starch and storage protein mutants. A prominent elevation in Sh2 and Bt2 transcripts is associated with increases in the cognate proteins, albeit of a much lesser magnitude. The genetic lesion, at least for starch synthesis, is dependent on the presence of a BT2 or SH2 protein, respectively. The SH2 protein is present in wild-type amounts in blocked starch mutants, zein protein is greatly reduced (Barbosa and Glover, 1978). Clearly, this reduction in zein is not due to a lack of zein transcripts. Furthermore, the increased transcripts of the starch synthetic genes in 02- may be related to the increase seen in the non-zein proteins.

For example, the transcriptional activity of maize photosynthetic promoters is repressed by Suc, Glc, and acetate in maize protoplasts relative to a mannitol control (Sheen, 1990). More germane to our system, Koch et al. (1992) showed that Sh1 and Sus1 expression in maize root tips is modulated by sugar concentration, with each gene exhibiting a distinctive profile. Taken together, the evidence indicates that the effects of sugars may be related to the identity of the tissue as a source of or a sink for carbohydrates, as has been suggested by others. Genes involved in the production of Suc would be repressed by Suc and those involved in the production of starch from Suc would be stimulated. Such an explanation may explain part of the global results presented here in the starch and storage protein mutant backgrounds.

**Endosperm Storage Product Genes Are Coordinately Regulated**

Coordinate gene expression is required for the successful development of any organism, including the maize kernel. Our data show that expression of many genes involved in endosperm development is sensitive to perturbations in developmental or biochemical processes seemingly not directly related to the affected gene. We show that transcript accumulation of genes involved in starch and storage protein synthesis is elevated when the synthesis of these end products is impaired. The alteration is at the transcriptional level, is dependent on the severity of the lesion, and does not alter the expression of the affected genes to the same degree. The pronounced elevation in Sh2 and Bt2 transcripts is associated with increases in the cognate proteins, albeit of a much lesser magnitude. The genetic lesion, at least for starch synthesis, can be simulated by alterations in the concentration of Suc fed to the kernel. Elevation in sugars or alteration in osmotic conditions, rather than the depletion in starch, apparently is involved in the signal transduction pathway.

The identification of the primary signal triggering this response awaits further investigation. Seemingly, the primary signal is not Suc, since transcripts are altered in mutants (e.g. o2 and azc) not known to significantly alter sugar content. It is interesting to note that o2- kernels dry down more slowly than their wild-type counterparts on segregating and otherwise isogenic developing ears (M. Lopes and B. Larkins, personal communication). Slower kernel dry-down is a well-known pleiotropic effect of the starch mutants, presumably because of the ability of the sugars to increase the moisture-holding capacity. Perhaps transcription of these storage-product genes is affected by the osmotic potential of the endosperm.

Although zein transcripts are elevated in the severely blocked starch mutants, zein protein is greatly reduced (Barbosa and Glover, 1978). Clearly, this reduction in zein is not due to a lack of zein transcripts. Furthermore, the increased transcripts of the starch synthetic genes in 02- may be related to the increase seen in the non-zein proteins.

Although SH2 and BT2 proteins are elevated in some mutants primarily at the latter stages of development, the extent of the increase is much less than that noted for their transcripts. This result is unexpected, since AGP activity shows a linear increase with the number of functional SH2 or BT2 alleles (Hannah and Nelson, 1975). Collectively, these data point to mechanisms regulating protein amount that are, or can become, independent of transcript abundance. This is similar to a case described for the Shl gene in which transcripts increase in response to anaerobiosis without a concomitant increase in protein (Talliercio and Chourey, 1989). However, it contrasts with the anaerobic induction of Suc synthase transcription and translation in rice (Ricard et al., 1990).

Aside from the complexity mentioned above, our data also suggest that the rates of SH2 or BT2 protein turnover are dependent on the presence of a BT2 or SH2 protein, respectively. The SH2 protein is present in wild-type amounts in

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### Table V. Relative Sh1, Sh2, Bt2, and S zein transcripts in wild-type kernels grown on different concentrations of Suc or mannitol

<table>
<thead>
<tr>
<th>Treatment (m)</th>
<th>Transcript</th>
<th>Sh1</th>
<th>Sh2</th>
<th>Bt2</th>
<th>S zein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suc</td>
<td></td>
<td>0.53</td>
<td>0.34</td>
<td>0.25</td>
<td>0.44</td>
</tr>
<tr>
<td>0.06</td>
<td></td>
<td>0.74</td>
<td>0.66</td>
<td>0.62</td>
<td>0.54</td>
</tr>
<tr>
<td>0.60</td>
<td></td>
<td>0.72</td>
<td>0.91</td>
<td>1.01</td>
<td>0.37</td>
</tr>
<tr>
<td>0.90</td>
<td></td>
<td>0.72</td>
<td>0.91</td>
<td>0.77</td>
<td>0.46</td>
</tr>
<tr>
<td>Mannitol</td>
<td></td>
<td>0.66</td>
<td>0.52</td>
<td>0.37</td>
<td>0.47</td>
</tr>
<tr>
<td>0.06</td>
<td></td>
<td>0.91</td>
<td>0.81</td>
<td>0.80</td>
<td>0.87</td>
</tr>
<tr>
<td>0.60</td>
<td></td>
<td>0.66</td>
<td>0.52</td>
<td>0.39</td>
<td>0.65</td>
</tr>
<tr>
<td>0.90</td>
<td></td>
<td>0.55</td>
<td>0.34</td>
<td>0.35</td>
<td>0.52</td>
</tr>
<tr>
<td>Field 22 dpp</td>
<td></td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Field 26 dpp</td>
<td></td>
<td>0.73</td>
<td>0.72</td>
<td>0.75</td>
<td>0.81</td>
</tr>
</tbody>
</table>

For example, the transcriptional activity of maize photosynthetic promoters is repressed by Suc, Glc, and acetate in maize protoplasts relative to a mannitol control (Sheen, 1990). More germane to our system, Koch et al. (1992) showed that Sh1 and Sus1 expression in maize root tips is modulated by sugar concentration, with each gene exhibiting a distinctive profile. Taken together, the evidence indicates that the effects of sugars may be related to the identity of the tissue as a source of or a sink for carbohydrates, as has been suggested by others. Genes involved in the production of Suc would be repressed by Suc and those involved in the production of starch from Suc would be stimulated. Such an explanation may explain part of the global results presented here in the starch and storage protein mutant backgrounds.

The identification of the primary signal triggering this response awaits further investigation. Seemingly, the primary signal is not Suc, since transcripts are altered in mutants (e.g. o2 and azc) not known to significantly alter sugar content. It is interesting to note that o2- kernels dry down more slowly than their wild-type counterparts on segregating and otherwise isogenic developing ears (M. Lopes and B. Larkins, personal communication). Slower kernel dry-down is a well-known pleiotropic effect of the starch mutants, presumably because of the ability of the sugars to increase the moisture-holding capacity. Perhaps transcription of these storage-product genes is affected by the osmotic potential of the endosperm.

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Aside from the complexity mentioned above, our data also suggest that the rates of SH2 or BT2 protein turnover are dependent on the presence of a BT2 or SH2 protein, respectively. The SH2 protein is present in wild-type amounts in
14-dpp bt2- seeds; yet in 30-dpp bt2- kernels, it is greatly reduced relative to wild type and the other mutants. The identical pattern was seen with the BT2 protein in sh2-mutants. These observations are reminiscent of an Arabidopsis mutant (Lin et al., 1988) that lacks both subunits of AGP. Although this mutation may lie in a regulatory gene involved in AGP synthesis, as the authors suggest, it seems equally plausible that the mutation is in a structural gene and the subunit of the nonallelic, wild-type gene is unstable in the absence of the other subunit.

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