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Genes and cDNAs for starch-branching enzyme II (SBEII) have been isolated from libraries constructed from Aegilops tauschii and wheat (Triticum aestivum) endosperm, respectively. One class of genes has been termed wSBEII-DA1 and encodes the N terminus reported for an SBEII from wheat endosperm. On the basis of phylogenetic comparisons with other branching enzyme sequences, wSBEII-DA1 is considered to be a member of the SBEIIa class. The wSBEII-DA1 gene consists of 22 exons with exons 4 to 21 being identical in length to the maize (Zea mays) SBEIIb gene, and the gene is located in the proximal region of the long arm of chromosome 2 at a locus designated sbe2a. RNA encoding SBEIIa can be detected in the endosperm from 6 d after flowering and is at its maximum level from 15 to 18 d after anthesis. Use of antibodies specific for SBEIIa demonstrated that this protein was present in both the soluble and granule bound fractions in developing wheat endosperm. We also report a cDNA sequence for SBEIIa that could arise by variant transcription/splicing. A second gene, termed wSBEII-DB1, was isolated and encodes an SBEII, which shows greater sequence identity with SBEIIb-type sequences than with SBEIIa-type sequences. Comparisons of SBEII gene structures among wheat, maize, and Arabidopsis indicate the lineage of the SBEII genes.

In cereals starch makes up approximately 65% of the weight of the mature grain. The starch is produced in the amylplast of the endosperm by the coordinated action of a number of enzymes that include ADP-Glc PP (EC 2.7.7.27), starch synthases (EC 2.4.1.21), branching enzymes (EC 2.4.1.18), and debranching enzymes (EC 3.2.1.41 and EC 3.2.1.68) (Martin and Smith, 1995; Morell et al., 1995; Ball et al., 1996). Some of these enzymes can be detected within the starch granule (Denyer et al., 1995; Rahman et al., 1995; Yamamori and Endo, 1996).

There are two main components of starch: amylose (normally approximately 25% of the total starch by mass in wheat [Triticum aestivum]), which is essentially linear, and amylopectin (comprising the remaining 75%), which is highly branched. The branches are formed by α-1,6 linkages joining α-1,4 linked Glc chains. This branching requires the action of starch-branching enzymes of which there are two types in plants, starch-branching enzyme I (SBEI), and starch-branching enzyme II (SBEII). Both SBEI and SBEII are 88 kD in size in wheat (cv Rosella and cv Wyuna). It has been shown that maize (Zea mays) SBEII transfers longer chains than maize SBEIIb (Takeda et al., 1993). Furthermore, maize SBEI preferentially branches amylose; its rate of branching amylopectin is less than 10% of that of branching amylose (Guan and Preiss, 1994). In contrast the rate of branching amylopectin is approximately 6-fold higher for SBEII than for branching amylose (Guan and Preiss, 1994). These results indicate that branching enzyme isoforms have distinct roles in amylopectin synthesis.

In the dicots pea and potato, one form of SBEII has been described (Poulsen and Kreiberg, 1993; Burton et al., 1995). The gene for this enzyme in peas (variously designated as SBEI, or SBE B; see Burton et al., 1995) has been shown to be non-functional (Bhattcharaya et al., 1990) in the high amylose peas mutant that has a wrinkled appearance. In maize two forms of SBEII, SBEIIa and SBEIIb, have been separated by anion-exchange separation (Boyer and Preiss, 1978) and characterized (Singh and Preiss, 1985), and it has been shown that these closely related isoforms are...
encoded by different genes (Fisher et al., 1996a). The SBEIIa and SBEIIb genes are expressed predominantly in the leaves and endosperm, respectively (Gao et al., 1997). In rice (Oryza sativa), two isoforms of SBE II, IIa and IIb, are found in the endosperm, but IIb is the major isoform and unique to the endosperm (Yamanouchi and Nakamura, 1992). In barley (Hordeum vulgare), both SBEIIa and SBEIIb forms show comparable activities in the endosperm (Sun et al., 1997) but the IIb form is only expressed in the endosperm. In contrast to these examples, anion-exchange chromatography of wheat soluble extracts yielded a single peak of SBEII activity (Morell et al., 1997), and a single N-terminal sequence was obtained (Morell et al., 1997). Mutations in SBEIIb are known to lead to an increased proportion of amylose in maize and rice (Mizuno et al., 1993; Kim et al., 1998). In contrast mutations in SBEIIa or SBEI have not yet been reported, and this may be because they do not lead to a distinguishable or recoverable phenotype.

In maize, rice, and barley (Mizuno et al., 1993; Gao et al., 1997; Sun et al., 1998; GenBank accession no. E14743) two types of cDNA encoding closely related forms of SBEII have been identified. These correspond to the IIb (the major form in the grain) and the IIa forms of maize. The cDNA sequences for maize SBEIIa and the SBEIIb show 85% sequence identity over 80% of their lengths but diverge at the 5' and 3' ends (Gao et al., 1997). The complete gene sequence for maize SBEIIb has also been reported (Kim et al., 1998a). It consists of 22 exons spread over 17 kb of DNA. For barley, partial sequences of genes identified for the SBEIIa and SBEIIb forms have been reported (Sun et al., 1998). In wheat, Nair et al. (1997) reported one type of cDNA sequence (GenBank accession no. Y11282) and this encodes the N terminus of SBEII as reported by Morell et al. (1997). The other cDNA for wheat SBEII in the GenBank database (accession no. U66376) is also closely related to the sequence reported by Nair et al. (1997). It has not been clear whether the abundant form of SBEII found in the wheat endosperm, and represented by these cDNA sequences, is the counterpart to the SBEIIa or SBEIIb form of maize.

In this paper, we report the structure of a gene isolated from Aegilops tauschii (the donor of the D genome to wheat), which encodes the most highly expressed isoform from the soluble phase of the wheat endosperm. Phylogenetic comparisons show that this gene is an SBEIIa-type gene, and direct sequence comparison confirms that the gene is more closely related to the barley SBEIIa gene than the barley SBEIIb gene. The gene is designated wSBEII-DA1. We show by in situ hybridization that the wSBEII-DA1 (GenBank accession no. AF338432), which differs from Y11282, the cDNA previously reported for SBEIIa (Nair et al., 1997) in that it is missing exon 2 and a portion of exon 1 so that the N terminus reported by Morell et al. (1997) is not encoded. RNA encoding SBEIIa type polypeptides is present in the wheat endosperm from 6 d after anthesis, a period considerably earlier than that observed for SBEI (Rahman et al., 1999), which is in agreement with protein expression data previously reported (Morell et al., 1997). An antisera raised against the N-terminal sequence of SBEIIa reacted against polypeptides in both the soluble and granule fractions showing that SBEIIa was present in both these fractions. Hybridization with a sequence corresponding to positions 537 to 890 of cDNA1 indicated the presence of at least another SBEII-like gene in A. tauschii and one such gene was isolated. Sequence data from this second gene indicates that it is an SBEIIb class gene and is more similar to the barley SBEIIb gene than the barley SBEIIa gene. This gene from A. tauschii has been named wSBEII-DB1. The structures of SBEIIa and SBEIIb genes are compared with genes from maize and Arabidopsis and gene lineages deduced.

RESULTS

Isolation of cDNA and Genomic Clones Encoding the Major Form of SBEII in the Soluble Phase of the Wheat Endosperm

Screening of a wheat cv Rosella cDNA library prepared from endosperm (mid-stage of development) with the maize SBEI clone (Baba et al., 1991) at low stringency led to the isolation of two classes of positive plaques. One class hybridized strongly to the probe and encoded wheat SBEI (Rahman et al., 1997, 1999). The second class was weakly hybridizing. The clone with the longest insert from this second class was called cDNA1 and its sequence showed greater identity to SBEII than to SBEI-type sequences. The sequence of cDNA1 will be discussed in detail later.

cDNA1 was used to isolate genes for SBEII by screening approximately 5 × 10⁸ plaques from a library constructed from A. tauschii (Rahman et al., 1997), the D genome donor of wheat. The isolated clones, named F1, F2, F3, and F4, were purified and the DNA analyzed by restriction mapping and hybridization. The results were consistent with the same gene being isolated in these different clones. Hybridization of F1 to F4 with the 5' and 3' ends of cDNA1 indicated that F1 contained the 3' end but not the 5' end. Conversely, F2, F3, and F4 contained the 5' end but not the 3' end (data not shown). Sequencing of common fragments from F1 and F2 demonstrated that an identical sequence was contained within the two clones, and thus F1 and F2 were defined as a contig providing the structure of an SBEII gene that we have designated wSBEII-DA1. Sequence from the region corresponding to exon 2
(derived from the clone F2, Fig. 1) showed that wSBEII-DA1 encoded the N-terminal sequence of the SBEII protein reported by Morell et al. (1997).

Structure of the Gene

The sequence of wSBEII-DA1 showed high identity (95%–100% depending on the region compared) to both cDNA1 and a previously reported cDNA sequence for SBEII, Y11282 (Nair et al., 1997). We therefore used these cDNAs to define the intron-exon structure for wSBEII-DA1. (Fig. 1). The structure is complex, consisting of 22 exons, which vary in size from 43 to 384 bp and cover a region of 10.5 kb. The size of the introns vary from 83 bp to 1,019 bp.

The principal difference between cDNA1 and Y11282 lies in that cDNA1 is missing part of exon 1 and all of exon 2 such that it does not contain the N terminus sequence reported by Morell et al. (1997). However cDNA1 encodes a functional branching enzyme as it could complement Escherichia coli mutants lacking branching enzyme due to the glgB mutation (data not shown).

None of the 10 cDNAs we isolated from the library constructed from the cv Rosella contained the N terminus of SBEII (Morell et al., 1997); they all contained part of exon 1 but were missing all of exon 2. A cDNA library constructed from the cv Wyuna was also analyzed. Sequences encoding the N terminus were found in both of the clones isolated (data not shown) and the sequence is identical to that in Y11282. The reason for the discrepancy between the two sources of clones is not clear as the protein sequence reported by Morell et al. (1997) was obtained from the cv Rosella. It is possible that cDNA1 represents the product of an alternate splicing event from a wSBEII-DA1 type gene (see “Discussion”). However the putative protein product of cDNA1 has not been detected in the endosperm and all our data to date indicate that the sequence reported by Morell et al. (1997) represents the most abundant form of SBEIIa in the soluble phase of wheat endosperm.

Analysis of the promoter region of wSBEII-DA1 revealed the presence of motifs commonly associated with genes expressed in the endosperm. For example, the endosperm box motif (canonical sequence TGTA-AAG; Forde et al. (1985)) was observed at a position approximately 300 bp upstream of the translation initiation site. The GCN 4 box, said to regulate expression according to nitrogen availability (Muller and Knudsen, 1993), was not observed in the wSBEII-DA1 promoter. The SBEIIa promoter was further examined by the transformation of rice with constructs containing the SBEIIa promoter, the β-glucuronidase (GUS) gene (uidA), and a nos 3′-terminator region. GUS expression was in all cases only observed in the seed but not in roots, leaves, or stems (data not shown).

Chromosomal Location of wSBEII-DA1 Type Genes by Fluorescent in Situ Hybridization

Fluorescent in situ hybridization (FISH) was performed with F2, the genomic clone containing part of wSBEII-DA1. The F2 clone clearly hybridized to the proximal region of chromosome 2 (Fig. 2). The identity of the chromosome was verified by double labeling with pSc119.2, a repetitive sequence used for chromosome identification (Mukai et al., 1990). The location of wSBEII-DA1 on chromosome 2 was confirmed by hybridization analysis (data not shown) using nulli-tetra stocks of wheat cv Chinese Spring (Sears and Miller, 1985; see “Materials and Methods”) and a fragment containing exons 4 to 9 and introns 3 (part), 4 through 8, and 9 (part) as the probe (Fig. 1). This location is consistent with the locus identified as Xwye1922(Sbe) in the genetic maps for wheat chromosome 2 (Sharp, 1997).

Number of wSBEII D1-Type Genes in Wheat and Isolation of a Second Type of SBEII Gene

Hybridization of A. tauschii DNA (cut with four different restriction enzymes) with a probe encoding Figure 1. Schematic representation of the intron-exon structure of wSBEII-DA1 (SbeIIa wheat) and comparison with the structures of genes for SBEIIb from maize (GenBank accession no. AF072725), SBE2.1 from Arabidopsis (GenBank accession no. AJ000497), SBE2.2 from Arabidopsis (GenBank accession no. AL162506.1), SBEI from A. tauschii (GenBank accession no. AF076680), and glycogen branching enzyme from E. coli (GenBank accession no. M13751). Lines connect five conserved residues (at positions 345, 349, 403, 448, and 526 of the glycogen branching enzyme; for clarity conserved residues at positions 405 and 525 are not shown) found in all members of the α-amylase superfamily (Svensson, 1994). The black boxes correspond to exons, the regions represented by a horizontal line to introns.
exons 4 to 9 and introns 4 to 8 and part of intron 9 (Fig. 1) revealed only one strongly hybridizing band and several very faint bands (Fig. 3a). This suggests that there is only one gene for SBEII in diploid *A. tauschii* and probably three genes for SBEII in the entire hexaploid wheat genome. However, hybridization with a probe that corresponds to only exons 5 to 9 (by amplifying positions 537–890 of cDNA1 to use as probe) leads to a much more complex pattern that cannot be explained by only one gene type for SBEII (Fig. 3b). We therefore used this probe to re-screen the genomic library from *A. tauschii*, and two clones were isolated that contained genes that were different to *wSBEII-DA1* both by PCR amplification of selected regions and by restriction mapping (data not shown). These clones have been designated G1 and G2 and have been shown to contain identical genes by sequencing (see below).

The sequence from a part of G1 showed 70% sequence identity over 320 bases with the sequence for *wSBEII-DA1* when the two sequences were compared using the program FASTA. However, the same sequence showed 89% sequence identity over 406 bases when compared with af064563, the sequence for the barley SBEIIb gene reported by Sun et al. (1998). On the other hand, *wSBEII-DA1* showed 70% sequence identity over 150 bases to af064563 but 90% over 1,586 bases to af064562, the barley SBEIIa sequence. We conclude therefore that *wSBEII-DA1* is an SBEIIa type gene and the gene contained in G1 is of an SBEIIb type. We have termed this SBEIIb type gene *wSBEII-DB1*. *wSBEII-DB1* is currently being sequenced.

Additional support for classifying *wSBEII-DA1* as an SBEIIa-type gene is provided by Figure 4, which shows the comparison of approximately the first 250 amino acids encoded by IIa- and IIb-type sequences from a number of sources using the program PileUp (Genetics Computer Group, Madison, WI; gap creation penalty of 12 and gap extension penalty of 0.1). The phylogenetic relationship of the entire deduced amino acid sequence of SBEs from diverse sources is shown in Figure 5 using the same conditions for PileUp.
Expression of \textit{\textit{w}SBEII-DA1}-Type Genes

RNA from developing wheat endosperm was hybridized to probes prepared from the extreme 3' end of cDNA1. This region does not hybridize to \textit{w}SBEII-DB1 or to SBEI-type sequences and is therefore specific to \textit{w}SBEII-DA1 type sequences. Hybridization to a RNA transcript of approximately 2.9 kb could be detected from 6 d after anthesis, which is considerably earlier than the hybridization reported for SBEI-type sequences (Baga et al., 1999; Rahman et al., 1999). The maximum expression (relative to ribosomal RNA standards) was in the period of 15 to 18 d after anthesis (Fig. 6). No RNA hybridizing to the \textit{w}SBEII-DA1 probe could be detected in the grain before anthesis or in leaves (data not shown).

Location of SBEIIa in the Endosperm

Antiserum was raised against the N terminus sequence of SBEIIa and evaluated for its specificity by testing against extracts prepared from \textit{E. coli} expressing polypeptides containing the N terminus of SBEIIa or the putative N terminus of the \textit{w}SBEII-DB1 gene product, respectively. The antiserum cross-reacted only with the \textit{E. coli} extract expressing SBEIIa N terminus (data not shown).

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The antiserum was used to probe extracts made from wheat endosperm mid-way through development. Using native PAGE, a single polypeptide was found to react in the soluble fraction (Fig. 7, lane a). If SDS-PAGE was used then a single reactive band of 88 kD was found to react in the granule-bound fraction (Fig. 7, lane c), and bands at 88 kD and at approximately 55 kD were found to react in the soluble fraction (Fig. 7, lane d). However, the band at 55 kD also reacted with preimmune serum and other antisera tested and is thus not due to a specific reaction. These results demonstrate that SBEIIa is present in both the soluble- and granule-bound fractions in the developing grain and indicate that the products of the three genomes co-migrate in gel electrophoresis.

DISCUSSION

In this paper we have described an SBEII gene, wSBEII-DA1, from the D genome donor of wheat, A. tauschii (Kawasaki et al., 1993; Kim et al., 1998b; Baga et al., 1999; Rahman et al., 1999). All the exons in wSBEII-DA1 are less than 400 bp in length and 18 of them are under 150 bp in length. In contrast, an SBEI gene from A. tauschii (wSBEI-D4) contains 14 exons with exon 6 being over 900 bp in length (Rahman et al., 1999). In wSBEI-D4, the central region corresponds to exon 6, and this contains the seven conserved amino acid positions found in all amylase enzymes (Svensson, 1994). In wSBEII-DA1, however these conserved amino acids are found in exons 11, 12, 13 (two), 14, and 16 (two). It is clear that the exon-intron structure of SBEI and SBEII genes are very different, although the enzymes themselves share similar molecular weights and approximately 65% sequence identity over the central third of the molecules at the amino acid level.

It is of interest to compare the wheat wSBEII-DA1 gene with that reported for maize SBEIIb (Kim et al., 1998a). The maize IIb gene also contains 22 exons but is much longer with the exons spread over 16.9 kb (compared with approximately 10.5 kb for wSBEII-DA1). However, the exon structure is strikingly similar with exons 4 through 21 being of identical length in the two species (Table I). This conservation is all
the more remarkable for the fact that the wheat wSBEII-DA1 gene encodes the IIa isoform and maize gene the IIb isoform. It would be of interest to compare the structure of the IIb isoform. It would be of interest to compare the structure of wSBEII-DA1 with the structure of the maize SBEIIa gene, but the maize SBEIIa gene has not yet been reported. We have previously observed variation in intron length between rice and wheat when comparing starch synthase I genes, although the size of the exons was identical (Li et al., 1999).

The classification of wSBE II-DA1 as an SBEIIa-type gene deserves comment. The polypeptide it encodes is the predominant SBEII isoform in the soluble phase of the wheat endosperm. This would lead to the expectation that wSBEII-DA1 encodes an SBEIIb-type polypeptide by analogy to maize where the SBE IIb form is the predominant form in the soluble phase (as measure by phosphorylation stimulation) and shows 92% sequence identity to intron 3 in the wheat SBE II-DA1 gene. In contrast there is no significant sequence identity to intron 3 in the reported sequence for barley SBE IIb (accession no. af064563), although the corresponding region of the gene is covered. It is interesting that in barley (in contrast to maize) the relative activities of SBEIIa and SBEIIb in the endosperm (as measure by phosphorylation stimulation) appear to be comparable with each other (Sun et al., 1997). Thus the situation in barley appears to be in between the two extremes of wheat (SBEIIa predominant in soluble phase) and maize (SBEIIb predominant in soluble phase). The maize SBE IIa gene and rice SBEIIa and SBE IIb genes have not yet been reported.

We have also compared the wheat and maize genes for SBEII with those for Arabidopsis. Two genes for SBEII from Arabidopsis are currently in the database. One, the gene for Arabidopsis SBE2.1 (GenBank accession no. AF072725) consists of 18 exons. Exons 4 through 16 are identical in length to that of maize and wheat SBEII (Table I). The second gene, for Arabidopsis, SBE2.2 (GenBank accession no. AL162506), like the maize and wheat SBEII genes, consists of 22 exons with exons 4 through 21 being of identical length to those of maize and wheat (Table I). The large exon 17 in SBE2.1 from Arabidopsis shows 70% to 80% sequence identity to the sequences of exons 17 to 21 from the Arabidopsis SBE2.2 gene. It is tempting to speculate that in a common ancestor of both monocots and dicots the original SBEII gene became duplicated. One lineage produced the Arabidopsis SBE2.1 gene type with 18 exons. The other lineage (represented by the Arabidopsis SBE2.2 gene and maize IIb and wheat IIa genes) was further fragmented to contain 22 exons. Sometime beyond this divergence between the two SBEII gene types the division of monocots and dicots took place and there was further duplication and divergence in the monocots to produce the IIa and IIb type genes. This speculative scenario raises the question of whether there is an SBEII gene of the Arabidopsis SBE2.1 type in monocots. It is possible that the probe that we found to hybridize to genes for both the SBEIIa and SBEIIb forms from wheat (see earlier) was not capable of hybridizing to the wheat gene that is the equivalent of the Arabidopsis SBE2.1 type-gene sequence.

In situ hybridization using the genomic clone F2 shows that the gene wSBEII-DA1 is located in the proximal region of the long arm of chromosome 2. This agrees with the results reported by Devos et al. (1993) who mapped an SBE sequence to chromosome 2 in wheat. Sun et al. (1998), similarly, located the barley SBEIIa gene to chromosome 2 (wheat homolog table: Comparison of exon lengths among SBE II genes

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* Arabidopsis SBE2.1 (Genbank accession no. AJ000497), Arabidopsis SBE2.2 (Genbank accession no. AL162506), wheat SBE IIa (this paper), and maize SBEIIb (Genbank accession no. AF072725). b The sequence in exon 17 of the Arabidopsis SBE2.1 gene shows high levels of identity with the sequence in exons 17 to 21 of the Arabidopsis SBE2.2 gene. c Exon 18 of the Arabidopsis SBE2.1 gene.
also chromosome 2). For SBEIIb, Sun et al. (1998) located the gene on barley chromosome 5 (wheat homolog chromosome 1), however Harrington et al. (1997) have predicted a location on wheat chromosome 6 on the basis of synteny with rice. In maize the ae mutation (due to a lesion in SBEIIb) has been located to maize chromosome 5 (Neuffer et al., 1997), which also could be consistent with a location on chromosomes 1, 4, or 6 of wheat. The location of wSBEII-DB1 thus clearly needs to be determined.

A number of genes that are expressed in the endosperm have been isolated. Forde et al. (1985) first described an “endosperm motif” (TGTAAG) that was common to storage protein genes expressed in the endosperm. Later Muller and Knudsen (1993) refined the endosperm motif as consisting of two elements: the endosperm box and the GCN4 element and hypothesized that in the archetype storage protein promoter the endosperm box determines tissue specificity and the GCN4 box responds to the availability of nitrogen. Our earlier analysis of promoters for starch biosynthetic enzymes (Li et al., 1999; Rahman et al., 1999) revealed that these promoters do contain an endosperm box motif (although often imperfect) but none contains the GCN4 box. In this paper the gene for SBEII has been described; this also contains an imperfect endosperm box motif but does not contain the GCN4 box. This is perhaps not surprising as storage protein synthesis is increased with increasing nitrogen availability but starch biosynthesis does not respond to nitrogen availability after a threshold is reached (e.g. Bruckner and Morey, 1988).

We have investigated the expression pattern for wSBEII-DA1 type of genes in wheat. In contrast to the situation in maize, where the IIa form was preferentially expressed in the leaves (Gao et al., 1996) and relatively weakly in the endosperm, we find that RNA for the wheat IIa form is preferentially expressed in the endosperm, reaching a peak 15 to 18 d after anthesis under our conditions (Fig. 6). Analysis of GUS expression in the endosperm of transgenic rice and barley plants also suggests that the promoter in wSBEII-DA1 is endosperm specific. However, it is possible that the use of more sensitive assays would allow the detection of very low levels of wSBEII-DA1 in other tissues. An antiseraum specific to the IIa form demonstrated that the polypeptide was present in both the granule and soluble phases of the endosperm. For maize, the IIb form is the predominant form in the endosperm soluble phase and it has not been reported whether it is also found in the granule. These results indicate important differences in the specificity and level of expression of individual branching enzyme genes between wheat and maize.

In this paper we have reported the sequence of cDNA1. It is very similar to Y11282 except that it is missing the sequences corresponding to parts of exon 1 and all of exon 2 of wSBEII-DA1 and consequently does not encode the reported N terminus for SBEII (Morell et al., 1997). Nevertheless this truncated polypeptide is capable of complementing a branching enzyme II mutation in glycogen biosynthesis in E. coli (data not shown), although the level of expressed protein was too low for any comparison of the turnover number or kinetic properties of the truncated form with the non-truncated form. The possibility of alternative transcription/splicing has been raised for a number of cereal biosynthetic enzymes such as ADP-Glc PP (Thorbjornsen et al., 1996), starch synthase (Baba et al., 1993), and SBEI (Baga et al., 1999; Rahman et al., 1999), and the phenomenon and its significance in the expression of the wSBEII-D1 gene require further investigation.

Investigation of branching enzyme genes from across a range of species indicates that there are at least two genes in dicots (SBEI and SBEII, or SBE B and SBE A, depending on the nomenclature system used) and three genes in the cereals (SBEI, SBEIIa, and SBEIIb). In wheat, we have previously shown that there are multiple SBEI genes in each genome, including at least one gene that encodes the 88-kD form expressed in the wheat endosperm and a number of pseudogenes. A recent report presents evidence for an additional high M, form of SBEI (Baga et al., 2000), which may be encoded by genes previously designated as pseudogenes. In this paper we describe the characterization of a gene for SBEIIa, wSBEII-DA1, and report the cloning and partial characterization of a gene for SBEIIb, wSBEII-DB1. Neither gene appears to be present in the wheat genome in pseudogene arrays such as are found for SBEI. Comparison with Arabidopsis gene sequences and gene organizations suggests that the monocot SBEII genes are most closely related to the Arabidopsis SBE2.2 gene and that it is likely that the SBEIIa and SBEIIb genes arose in monocots through a gene duplication event that post-dates the divergence of dicots from monocots. The requirement for gene duplication, such that dicots contain at least two SBE genes and monocots contain three SBE genes, remains a subject for further investigation. Mutation or down-regulation of SBEI in a wild-type background has minimal impact on starch structure, however, in potato down regulation of SBEI in the presence of SBEII down regulation leads to further increases in amylose content over SBEII down regulation alone. In monocots, high amylose phenotypes reported to date result from mutation or down regulation of SBEIIb. These observations suggest that while each of these enzymes possesses branching enzyme activities, albeit with altered specificities when exposed to defined substrates in vitro, the physiological action of each of these enzymes in the developing monocot endosperm is distinct and highly defined.
MATERIALS AND METHODS

Plant Material

*Agrostis tauschii*, CPI 110799, was used for the construction of the genomic library. Previously this accession has been shown to be most like the ancestral D genome donor of wheat (*Triticum aestivum*) based on the conserved linkage of genetic markers (Lagudah et al., 1991). Wheat cv Rosella and cv Wyuna were used for the construction of different cDNA libraries. Nulli-tetrasom analysis to locate the gene for SBEIIa, was carried out using stocks of cv Chinese Spring that were nullisomic for a chromosome pair from one genome but tetrasomic for the homeologous chromosome pair from another genome (Sears and Miller, 1985).

Construction and Screening of cDNA and Genomic Libraries

The construction of the cDNA and genomic libraries described in this paper has been described in Rahman et al. (1997, 1999) and in Li et al. (1999). Conditions for library screening were hybridization in 25% (v/v) formamide, 5 × SSC, 0.1% (v/v) SDS, 10× Denhardt’s solution, 100 μg mL⁻¹ salmon sperm DNA at 42°C for 16 h followed by washing in 2× SSC, 0.1% (v/v) SDS at 65°C for 3 × 1 h.

Isolation of cDNAs and Genomic Clones for SBEII

The maize (*Zea mays*) SBEII (Baba et al., 1991) probe was used to probe the cDNA library from cv Rosella at low stringency. One class of weakly hybridizing sequences were found to show high identity to rice (*Oryza sativa*) SBEII sequences and the longest insert in this group was called cDNA1. The 5’ end of cDNA1 (positions 1–300) was used to screen a second cDNA library prepared from the cv Wyuna (Li et al., 1999).

A genomic library constructed from *A. tauschii* was screened with cDNA1 clone and four positive plaques were purified and designated F1 to F4. The sequence from positions 537 to 890 of cDNA1 was amplified by PCR and used to screen the *A. tauschii* library again. Clones isolated from this second screening that contain a different gene from that contained in F1 to F4 are referred to as G1 and G2.

Sequencing and Sequence Analysis

Sequencing was performed on an ABI 377 sequencer using dye terminator technology following protocols recommended by the manufacturer. Sequences were analyzed using the GCG suite of programs (Devereaux et al., 1984).

In Situ Hybridization

In situ hybridization with the genomic clone F2 was performed on chromosome squashes from *A. tauschii* and wheat exactly as described in Turner et al. (1999).

Northern-Blot Analysis

RNA from endosperm at different developmental stages was obtained from wheat grown in the greenhouse as described in Li et al. (1999). RNA was extracted by the method of Higgins et al. (1976), separated on denaturing formamide gels, and blotted onto Hybond N⁺ paper (Amersham, Buckinghamshire, UK), essentially as described in Maniatis et al. (1982). Probes were prepared from the extreme 3’-untranslated region of cDNA1 (bases 2,450–2,640) by PCR using the following protocol: 94°C, 2 min, 1 cycle; 94°C, 30 s, 55°C, 30 s, 72°C, 30 s, 36 cycles; 72°C, 5 min, 1 cycle; 25°C, 1 min, 1 cycle.

Southern-Blot Analysis

DNA was isolated from leaves of *A. tauschii* using established protocols (Maniatis et al., 1982). Approximately 10 μg of DNA was transferred to nylon membranes (Hybond N⁺, Amersham) and hybridized to indicated probes under conditions identical to those used for library screening above.

Gel Electrophoresis and Immunoblotting

SDS-PAGE, non-denaturing PAGE, and immunoblotting were carried out as described previously (Morell et al., 1997). A peptide was synthesized with a sequence from the N-terminal region of SBEII from wheat endosperm, AASPGKVLVPDGESDDL, followed by an additional C-terminal GC dipeptide. This peptide was linked to keyhole limpet hemocyanin and used as antigen according to methods described in Morell et al. (1997). The resulting antibodies were designated anti-wheat SBEIIa.

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LITERATURE CITED


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CORRECTIONS


In two places, the sequence “pSc119.2” was mentioned instead of “pAs1.” On p 1316, the correct sentence should be “The identity of the chromosome was verified by double labeling with pAs1, a repetitive sequence used for chromosome identification (Mukai et al., 1990).” On p 1317 in the legend to Figure 2, the correct sentence should be “FISH of a wSBEII-DA1 probe (white dots) and a repetitive DNA sequence probe (pAs1, pink dots) to A. tauschii chromosomes (main photograph and lower insert) and wheat chromosomes (upper insert).”


Dinges, J.R., Colleoni, C., Myers, A.M., and James, M.G. Molecular Structure of Three Mutations at the Maize sugary1 Locus and Their Allele-Specific Phenotypic Effects.

The authors of this article were mistakenly omitted from the Author Index in Vol. 125, No. 3.