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Isolation of cDNAs encoding guanine nucleotide-binding protein β-subunit homologues from maize (ZGB1) and *Arabidopsis* (AGB1)

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ABSTRACT **We have isolated cDNAs from maize (ZGB1) and *Arabidopsis* (AGB1) encoding proteins homologous to β subunits of guanine nucleotide-binding protein (G protein). The predicted *ZGB1* and *AGB1* gene products are 76% identical to each other and 41% or more identical to animal G protein β subunits. Both predicted proteins contain seven repeats of the so-called "WD-40" motif, where WD is Trp-Asp. RNA blot analysis indicates that ZGB1 mRNA is present in the root, leaf, and tassel and that AGB1 mRNA is expressed in the root, leaf, and flower. DNA blot hybridizations indicate that maize and *Arabidopsis* genomes contain no other genes that are highly similar to *ZGB1* and *AGB1*, respectively, suggesting that the newly isolated G protein β-subunit homologues are likely to have unique functions. Furthermore, these G protein β-subunit homologues are conserved among other plant species and may play important role(s) in plant signaling.**

In animal and simple eukaryotes, heterotrimeric guanine nucleotide-binding proteins (G proteins), which consist of α, β, and γ subunits, play important roles in the transmission of signals such as hormones and light from membrane receptors to different effectors, including adenylyl cyclases, ion channels, and phospholipases (1). Upon interaction of the inactive GDP-bound G protein with an activated receptor, the GDP is replaced by a GTP and the βγ complex dissociates from the GTP-bound α subunit. Until recently, the GTP–α subunit complex was thought to confer the G-protein specificity, while the role of the βγ complex in signal transduction was controversial (2). There are four β and six γ subunits in mammals (3), and recent studies indicate that the βγ complex is also able to interact directly with effectors, such as adenylyl cyclases, K^+ channels, and phospholipase A_2 (4, 5). Furthermore, in some cases the βγ complex is involved in receptor recognition (6, 7).

In plants, biochemical studies have suggested G protein involvement in a number of pathways (8), such as the control of K^+ channel opening in guard cells and mesophyll cells (9, 10) or the transmission of red and blue light-induced signals (11, 12). Furthermore, the cloning of *Arabidopsis* gene *GPA1* encoding a G protein α subunit designated GPα1 and its tomato homologue has provided molecular evidence for the existence of G protein-mediated signaling pathway(s) in plants (13, 14). The reports of G protein involvement in a number of different cellular functions raise the possibility that GPα1 may be involved in more than one signaling pathway. It is possible that G protein β subunit(s) might also play a direct role in signaling in plants.

In this report, we describe the cloning of the maize ZGB1 and *Arabidopsis* AGB1 cDNAs encoding proteins that share >41% identity with animal G protein β subunits.§ This level of homology is greater than that between a known yeast G protein β subunit and animal ones (15). ZGB1 and AGB1 may represent an additional type of G protein β subunit that is conserved in flowering plants and expressed in roots, leaves, and flowers.

MATERIALS AND METHODS

Maize Subtracted Library. A maize tassel cDNA library in pCDNAII (Invitrogen) and a maize ear shoot cDNA library in λ-Uni-ZAP (Stratagene) were provided by M. Albertsen and G. Huffman, respectively, of Pioneer Hi-Bred International. Biotinylated RNA was generated *in vitro* from the ear shoot library by using the Gemini Riboprobe system (Promega) with the manufacturer's protocol modified to include 1 mM biotin-11 rUTP (Enzo Diagnostics, instead of photobiotin rUTP) and an increased rUTP concentration (1 mM) in the transcription reaction. A subtracted tassel cDNA library was prepared by hybridization of the biotinylated RNAs with single-stranded DNA from the tassel library as described (16). The second strand of the nonsubtracted DNA molecules was synthesized with Klenow as described (17). One of the obtained clones was designated pPHP2541.

5′ Rapid Amplification of cDNA Ends (RACE) for PCR. 5′ RACE primer extension was performed by using the 5′ RACE system (GIBCO/BRL) with leaf and tassel poly(A)$^+$ RNA and the oligonucleotide 5′-GATATCCACAGCCTA-CAGTTG-3′ derived from the sequence of the pPHP2541 cDNA insert. The pPHP2541-derived nested primer 5′-GTATTTGATGAGTTGATGGAC-3′ and the provided anchor primer were used for PCR amplification with *Taq* I polymerase (Perkin–Elmer). A clone containing a 0.6-kb PCR product was named pPHP3573.

Library Screening, Subcloning, and Sequence Analysis. A λ-Uni-ZAP maize tassel cDNA library was screened for a full-length cDNA by using standard conditions and a ^{32}P-labeled 0.9-kb insert from pPHP2541 as a probe. A λYES cDNA library from *Arabidopsis thaliana* (ref. 18; a gift from J. Mulligan) was screened with ^{32}P-labeled 0.9-kb and 0.6-kb maize cDNAs from pPHP2541 and pPHP5373, respectively. cDNAs were subcloned into the Promega vector pGEM-7Zf(+) for sequencing of both strands.

Southern and Northern Blot Hybridizations. Genomic DNA isolated from *Arabidopsis* (Landsberg *erecta*), maize (inbred A632), broccoli, carrot, and green bean (from a local store) were analyzed by DNA blot hybridization with a portion of either AGB1 or ZGB1 cDNA labeled with ^{32}P. After hybridization, membranes were washed at room temperature for 10 min in 2× SSC (1× = 0.15 M NaCl/0.015 M sodium citrate, pH 7) containing 0.1% SDS and then twice at 65°C for 10 min, first in 1× SSC containing 0.1% SDS and second in 0.1× SSC containing 0.1% SDS for high stringency. Alternatively, membranes were washed at low stringency in 6× SSC

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Abbreviations: G protein, guanine nucleotide-binding protein; ORF, open reading frame; RACE, rapid amplification of cDNA ends.

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§The sequences reported in this paper have been deposited in the GenBank data base (accession nos. AGB1, U12232; ZGB1, U12233).

containing 0.1% SDS for 10 min at room temperature, then in 6× SSC containing 0.1% SDS at 50°C for 10 min, and finally in 4× SSC containing 0.1% SDS at 50°C for 10 min.

Total RNA from maize was isolated as described by Sharrock and Quail (19), and total RNA from *Arabidopsis* was isolated as described (20). The pellet was resuspended in TE buffer (10 mM Tris/1 mM EDTA, pH 8.0) containing 10 mM $MgCl_2$ and incubated with RQ1 DNase (10 units/ml; Promega) for 15 min at 37°C. After one extraction with 1:1 (vol/vol) phenol/chloroform, the RNA was precipitated with ethanol, stored at −20°C, pelleted, and resuspended in RNase-free water just before use. The RNA gel blots were hybridized with a ^{32}P-radiolabeled AGB1- or ZGB1-specific cDNA probe, and then washed, at high stringency.

RESULTS AND DISCUSSION

Isolation of *ZGB1* and *AGB1*. A subtracted maize tassel cDNA library was constructed for the purpose of isolating tissue-specific genes. During an initial screening of the subtracted library, several clones were selected at random from ≈1800 clones and sequenced. A putative open reading frame (ORF) in one of these clones, pPHP2541, encodes a peptide similar to the carboxyl portion of several animal G protein β subunits. This putative peptide is 49% identical and 72% similar to the last 152 amino acids from the human G protein β2 subunit (21). The gene represented by pPHP2541 was named *ZGB1*. Using RNA from maize leaf and tassels, 5′ RACE was then used to obtain additional coding sequence for the *ZGB1* gene. Nine 5′ RACE clones from tassel RNA and six from leaf RNA were sequenced and analyzed. An additional 481 bases of coding sequence was obtained from the 5′ RACE products. The overlapping nucleotide sequences of all of the 5′ RACE products were identical regardless of the tissue source of the RNA template. The additional sequence obtained from the 5′ RACE extended the homology of *ZGB1* with animal G protein β subunit genes but the sequence did not extend all the way to the translational start codon. A maize tassel cDNA library was then screened to obtain a full-length cDNA clone for *ZGB1*. Two candidate clones were isolated from 2×10^5 plaques; both of these appear to contain nearly full-length cDNA inserts of about 1.7 kb, differing in length by 12 nucleotides at the 5′ end. These two clones as well as pPHP2541 contain poly(A) tails but at different positions (Fig. 1). The larger clone, pPHP5729, was completely sequenced. The protein encoded by the longest ORF is designated ZGβ1 and has 380 amino acids (41,694 Da).

With two shorter maize clones (pPHP2541, 0.9 kb; and pPHP5373, 0.6 kb) as probes, a total of 15 putative positive clones were isolated after screening $\approx 1 \times 10^6$ plaques of an λYES *Arabidopsis* cDNA library. Sequence analysis indicated that 11 of these clones contain cDNAs from the same gene and were further analyzed. The longest of these clones, pMC1057, is ≈1.7 kb, while the others range in size from 0.7 to 1.5 kb. The gene represented by pMC1057 was named *AGB1*. Five of the clones contain the characteristic poly(A) tail, although at different positions (Fig. 2). The longest ORF (Fig. 2) is capable of encoding a protein, designated AGβ1, of 377 amino acids (40,960 Da).

Each of the 5′ untranslated regions of the ZGB1 and AGB1 cDNAs contains a short ORF about 65 bases upstream of the long ORF ATG, potentially coding a short peptide, Met-Arg-Gly-Ser-Ser and Met-Asn-Leu-Leu-Leu-Phe, respectively (Figs. 1 and 2). Short upstream ORFs have been observed in the *Arabidopsis* and tomato G protein α subunit genes (13, 14) and in several other plant (22), animal (23), and yeast genes (24). It is known that some upstream ATGs are involved in the translational regulation of specific genes in yeast (24) and in maize (25). Although it is not known if the small ORFs in the plant G protein β subunit cDNAs are translated, their similar sizes and positions suggest a potential functional role.

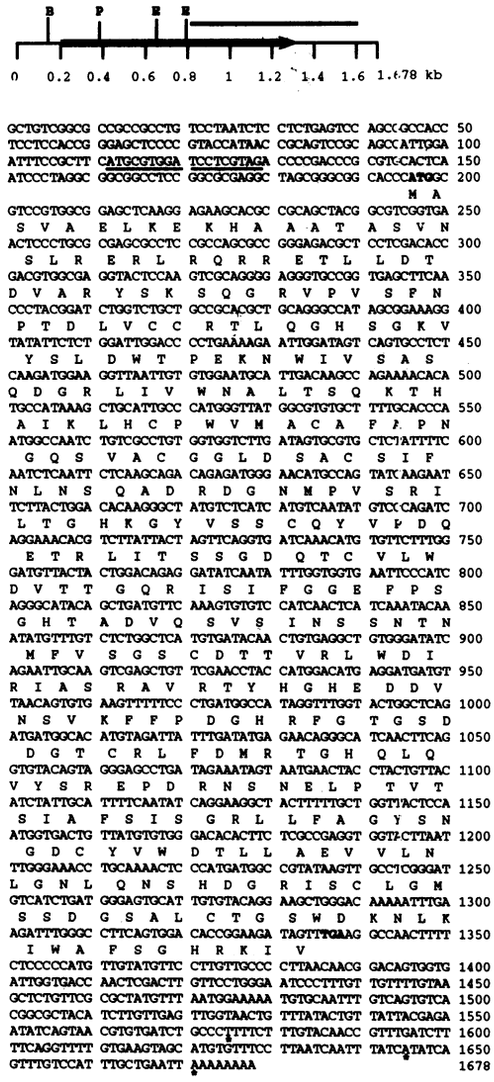

FIG. 1. Maize ZGB1 cDNA and sequence. (*Upper*) Restriction map of the ZGB1 cDNA with restriction sites B (*Bam*HI), P (*Pst* I), and E (*Eco*RI). The thick line and the thick arrow indicate the region used as the probe and the translated region, respectively. (*Lower*) ZGB1 cDNA and ZGβ1 amino acid sequences in single-letter code. Numbers refer to nucleotides. The ZGβ1 ORF is from position 197 to 1339, with the first and last codons in boldface letters. The upstream short ORF is underlined. The presumed polyadenylylation sites are indicated by asterisks below the sites. The sequence of the 5′ RACE products is from 337 to 847; the sequence of the pPHP2541 clone is from 788 to 1673.

ZGβ1 and AGβ1 Share Strong Sequence Similarities with Each Other and with Known G Protein β Subunits. ZGβ1 and AGβ1 are highly similar to each other, with 76% amino acid sequence identity and an additional 14% conservative replacements (Fig. 3). This high degree of similarity suggests that these proteins have the same function(s) in their respective plants. The proteins differ in length by 3 amino acids, and the amino acid sequence differences between them are scattered all along the sequences.

Sequence comparison of known G protein β subunits shows that they can be divided into three subfamilies (ref. 5; Fig. 4). The first subfamily includes all mammalian G protein β subunits, and one from each of squid, *Drosophila* brain (Gbb), and *Caenorhabditis elegans*; these proteins are ≈80% or more identical in sequence. The only known *Dictyostelium*

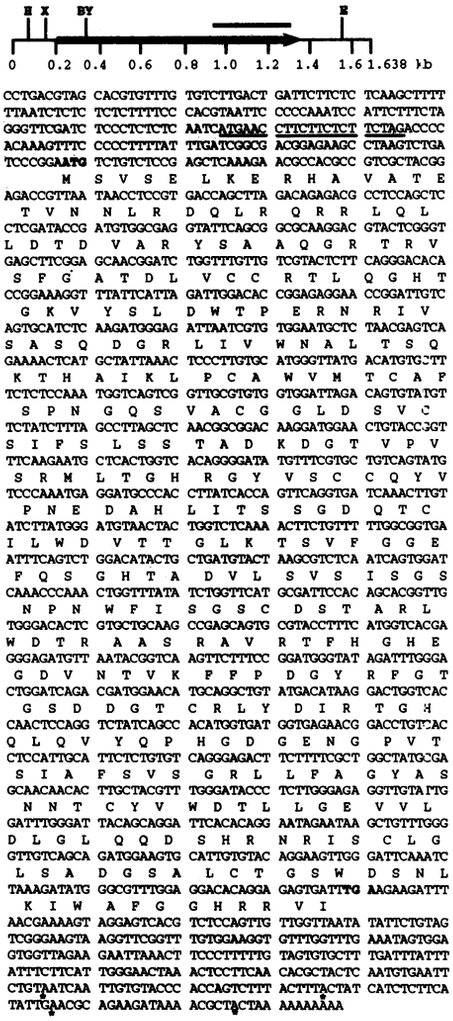

FIG. 2. *Arabidopsis* AGB1 cDNA and sequence. (*Upper*) Restriction map of the AGB1 cDNA with restriction sites H (*Hin*dIII), X (*Xba* I), BY (*Bst*YI), and E (*Eco*RI). Other symbols are as in Fig. 1. (*Lower*) AGB1 cDNA and AGβ1 amino acid sequences. The AGβ1 ORF is from position 209 to 1342, with the first and last codon in boldface letters.

β subunit is very similar to these animal ones (61–65% identity), and it can be considered as a member of this subfamily. Each of the two other β subunits, one from the *Drosophila* compound eye (Gbe) and the other from *Saccharomyces cerevisiae* (STE4), represent one subfamily (Fig. 4). Gbe and STE4 are ≈42% and 37% identical to those in the first subfamily, respectively, and are 28% identical to each other. Although STE4 is the most divergent β subunit and is ≈80 residues longer than the others, it has been shown to be a true G protein β subunit by functional studies (15). Alignments of the plant sequences with animal G protein β subunits indicate that ZGβ1 and AGβ1 share strong amino acid similarity (41–45% identity) with the members of the first subfamily. In particular, ZGβ1 and AGβ1 share 42% and 44% identity, respectively, to mammalian G protein β2 subunit and 43% and 45% to the *C. elegans* β subunit (Fig. 3). The fact that ZGβ1 and AGβ1 are more similar than STE4 to the animal G protein β subunits suggests that the plant proteins are also true G protein β subunits.

ZGβ1 and AGβ1 are ≈40 amino acids longer than the animal G protein β subunits. In the alignments of the plant and animal G protein β subunits (Fig. 3), most of the extra plant residues are distributed among several small regions of several residues each. In contrast, in the alignments between

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Hum. β          M-SELEQLRQ E------AEQ LRNQIRDARK ACGDSTLTQI TAGLD--PV-   40
C.Ele. β        M-SELDQLRQ E------AEQ LKSQIREARK SANDTTLATV ASNLE--PI-   40
AGβ1            M-SVSELKER HAVATETVNN LRDQLRQRRL QLLDTDVARY SAAQGRTRVS   49
ZGβ1            MASVAELKEK HAAATASVNS LRERLRQRRE TLLDTDVARY SKSQGRVPVS   50
Consensus       M.S...L... ........E. LR.Q.R..R. ...DT..A.. .......PV.   50

Hum. β          -GRIQMRTRR TLRGHLAKIY AMHWGTDSRL LVSASQDGKI IIWDSYTTNK   89
C Ele. β        -GRIQMRTRR TLRGHLAKIY AMHWASDSRN LVSASQDGKL IVWDSYTTNK   89
AGβ1            PGATDLVCCR TLQGHTGKVY SLDWTPERNR IVSASQDGRI IVWMALTSQK   99
ZGβ1            PNPTDLVCCR TLQGHSGKVY SLDWTPEKNW IVSASQDGRL IVWMALTSQK  100
Consensus        G.......R TL.GH..K.Y ...W...... .VSASQDG.L IVW...T..K  100
WD-40 motif     ..R...R... .*.GH...*. ......D.. .*.T.S.D... .*WD[...

Hum. β          VHAIPLRSSW VMTCAYAPSG NFVACGGLDN ICSIYSLKT- --REGNVRVS  136
C.Ele. β        VHAIPLRSSW VMTCAYAPSG SFVACGGLDN ICSIYSLKT- --REGNVRVS  136
AGβ1            THAIKLPCAW VMTCAPSPNG QSVACGGLDS VCSIFSLSST ADKDGTVPVS  149
ZGβ1            THAIKLHCPW VMACAFAPNG QSVACGGLDS ACSIFNLNSQ ADRDGNMPVS  150
Consensus        HAI.L...W VMTCA.AP.G ..VACGGLD. .CSI.SL... ..R.GNV.VS  150
WD-40 motif     ......... *......... ..*.....D. ...*..]..- -.R....R..

Hum. β          RELPGHTGYL SCCRFLDDN- -QIITSSGDT TCALWDIETG QQTVGF----  180
C.Ele. β        RELPGHTGYL SCCRFLDDN- -QIVTSSGDM TCALWDIETG QQCTAF----  180
AGβ1            RMLTGHRGYV SCCQYVPNED AHLITSSGDQ TCILWDVTTG LKTSVPGGEF  199
ZGβ1            RILTGHKGYV SSCQYVPDQE TRLITSSGDQ TCVLWDVTTG QRISIFGGEF  200
Consensus       R.L.GH.GY. SCC....D.. ...ITSSGD. TC.LWD..TG Q....F....  200
WD-40 motif     ..*.GH...* .......D.- -.*.T.S.D. ...*WD.[... ..........

Hum. β          -AGHSGDVMS LSLA-PDGRT FVSGACDASI KLWDVR-DSM CRQTFIGHES  227
C.Ele. β        -TGHTGDVMS LSLS-PDFRT FISGACDASA KLWDIR-DGM CKQTFPGHES  227
AGβ1            QSGHTADVLS VSISGSNPNW FISGSCDSTA RLWDTRAASR AVRTPHGHEG  249
ZGβ1            PSGHTADVQS VSINSSNTNM FVSGSCDTTV RLWDIRIASR AVRTYHGHED  250
Consensus         GHT.DV.S .S........ F.SG.CD... .LWD.R..S. ...TF.GHE.  250
WD-40 motif     .......*.. .......... *.....D... .*WD.R.-.. ....*.GH..

Hum. β          DINAVAFFPN GYAFTTGSDD ATCRLFDLRA DQELLMYSHD -NIICG----  272
C.Ele. β        DINAVAFFPS GNAFATGSDD ATCRLFDIRA DQELAMYSHD -NIICG----  272
AGβ1            DVNTVKFFPD GYRFGTGSDD GTCRLYDIRT GHQLQVYQPH GDGENG--P-  296
ZGβ1            DVNSVKFFPD GHRFGTGSDD GTCRLFDMRT GHQLQVYSRE PDRNSNELPT  300
Consensus       D.N.V.FFP. G..F.TGSDD .TCRLFD.R. ...L..YS.. .....G--.-  300
WD-40 motif     .*.......D ...*.T.S.D ....*WD.R. .......... ......----

Hum. β          ITSVAFSRSG RLLLAGY-DD FNCNIWDAMK GD---RAGVL -AGHDNRVSC  317
C.Ele. β        ITSVAFSKSG RLLFSGY-DD FNCNVWDSMR QE---RAGVI -AGHDNRVSC  317
AGβ1            VTSIAPSVSG RLLFAGYASN NTCYVWDTLL GEVVLDLGLQ QDSHRNRISC  346
ZGβ1            VTSIAPSISG RLLFAGY-SN GDCYVWDTLL AEVVLNLGNI QNSHDGRISC  349
Consensus        TS.AFS.SG RLLFAGY-.. ..C.VWD... .E.....G.L ...HDNR.SC  350
WD-40 motif     *......... .*.....-.D ....*WD..R ..---R...* -.GH...*..

Hum. β          LGVTDDGMAV ATGSWDSFLK IWN------- -  340
C.Ele. β        LGVTEDGMAV CTGSWDSFLK IWN------- -  340
AGβ1            LGLSADGSAL CTGSWDSNLK IWAPGGHRRV I  377
ZGβ1            LGMSSDGSAL CTGSWDKNLK IWAFSGHRKI V  380
Consensus       LG...DG.A. CTGSWDS.LK IW........ .  381
WD-40 motif     .....D...* .T.S.D.... *WD
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FIG. 3. Alignment of ZGβ1 (maize) and AGβ1 (*Arabidopsis*) with the following animal G protein β subunit sequences: Hum β (human β2 subunit; ref. 21), C Ele. β (*C. elegans*; ref. 26). The consensus sequence shows residues that are identical in at least three sequences. The repetitive WD-40 motif is shown (27). The motif is about 40 residues long, and generally starts with an arginine residue (R) and ends with a Trp-Asp (WD in single-letter code) dipeptide. If there are no conserved residues at those positions, the beginning or the end of the motif is indicated by "[" or "]", respectively. The second repeat is less conserved in all G protein β subunits and lacks the WD residues. Asterisks represent hydrophobic residues.

animal G protein β subunits and the yeast STE4 protein, most of the extra yeast amino acids are in two large regions of more than 30 residues, one at the N terminus and the other near the C terminus (15). Both the level of sequence identity and the distribution of extra residues in sequence alignments indicate that the plant G protein β subunits represent an additional subfamily of β subunits (Fig. 4). It is known that the yeast STE4 protein interacts with G protein α and γ subunits, receptors and effectors that are different from those that interact with the mammalian G protein β subunits (15, 34–36). By analogy, ZGβ1 and AGβ1 are likely to interact with one or more receptors and effectors that are different from those from mammals or yeast. The fact that the plant G protein β subunits constitute a previously unreported subfamily is reminiscent of the degree of relatedness between the α subunits from yeast or plants and those from animals; the yeast and plant G protein α subunits also form separate

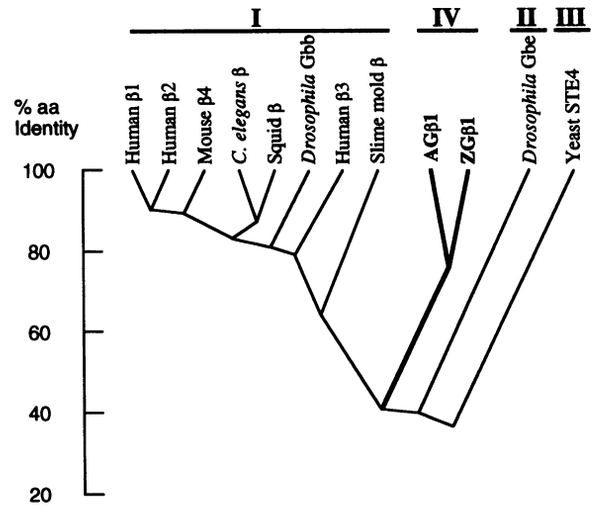

FIG. 4. Subfamilies of G protein β subunits (modified from Fig. 2 of Iñiguez-Lluhi *et al.*; ref. 5). Previously identified G protein β subunits are compared with the newly isolated plant proteins. Roman numerals I through IV indicate subfamilies. The average of percent amino acid (aa) identities is used to determine the position of each branch point. References for the proteins are as follows: human β1 (27), β2 (21), and β3 (28); mouse β4 (29); squid (30); *Drosophila* Gbb (31) and Gbe (32); *C. elegans* (26); *Dictyostelium* (33); and *S. cerevisiae* (STE4; ref. 15). ZGβ1 (maize) and AGβ1 (*Arabidopsis*) proteins are shown boldface. The two large extra regions of the STE4 protein (ref. 15; see text) are not included in the calculation of percent identity used for this tree; therefore, the actual similarity between STE4 and the others is less than that shown here.

subfamilies (8). The divergence of heterotrimeric G protein subunits among animals, plants, and yeast is in contrast with the situation for small G proteins, which are highly similar (>60% identity) and functionally interchangeable between the plant, animal, and yeast homologs (8).

Like all known G protein β subunits, ZGβ1 and AGβ1 both contain seven copies of a moderately conserved repeat called the "WD-40" motif (ref. 27; see Fig. 3), which contains conserved residues but also shows some degeneration. In addition to G protein β subunits, a large number of proteins from yeast to human have been shown to contain the WD-40 motif. They have very diverse functions, including cell cycle regulation, RNA splicing, regulation of Ras function, transcriptional repression, and actin binding (8, 37). These proteins have different sizes (from 318 to >800 residues) and various numbers (five to eight) of WD-40 repeats; furthermore, many of these proteins appear to be hybrid proteins with the WD-40 repeats at their C termini and an additional structural motif an their N termini. Fong *et al.* (27) suggested that the conserved repeats have evolved by duplication of a basic sequence of ≈40 amino acids. Most likely the WD-40 motif is involved in a general process, possibly playing a role in protein–protein recognition (38). In addition, it has been shown that the two functional regions of STE4 involved in pheromone signaling were located outside of the WD-40 repeats, suggesting that the role of the WD-40 repeats is not signaling (36).

WD-40 motif-containing proteins have also been isolated from plants; one of them is the *Arabidopsis* COP1 protein, which is 74.5 kDa in size and contains two zinc fingers in its N-terminal region; it was proposed to be a transcriptional repressor involved in photomorphogenesis (39). Two other plant WD-40 proteins have been identified: one is the *Chlamydomonas* Cblp protein (40), and the other is the gene product of *arc1* (an auxin-regulated gene) in tobacco (41). Because of the presence of WD-40 motifs, the Cblp and Arc proteins show low levels (about 25%) of sequence identity to G protein β subunits; therefore, they are not likely to be true G protein β subunits. However, Cblp and Arc share high levels (68% or more) of sequence identity with each other and with a WD-40 motif-containing protein from chicken (MHC12.3) that is encoded by a gene linked to the major histocompatibility complex (42), suggesting that they have similar yet unknown functions in their respective organisms (41, 43).

***ZGB1* and *AGB1* Are Single-Copy Genes That Are Conserved Among Different Species.** To determine if *ZGB1* and *AGB1* are members of a family of highly similar genes, we performed DNA blot hybridization of maize and *Arabidopsis* genomic DNA under low and high stringencies, using a portion of ZGB1 or AGB1 cDNA as probes (see Figs. 1 and 2). In either case, a single band was observed for *Arabidopsis* or maize DNA probed, respectively, with AGB1 cDNA (Fig. 5*A*, lanes 1–4) or with ZGB1 cDNA (Fig. 5*A*, lanes 8 and 9). These results indicate that both the *Arabidopsis* and the maize genomes lack other sequences that are very similar (>60% identity) to the cloned cDNAs. In other words, both *ZGB1* and *AGB1* are single-copy genes, unlike the G protein β subunit genes in mammals. Hybridization of a portion of AGB1 cDNA with the genomic DNA from various plants at low stringency detected homologous sequences in all of them, indicating that the gene *AGB1* is conserved among flowering plants (Fig. 5*A*, lanes 5–7). In addition, when the plant genome is tetraploid (broccoli and carrot, both hybrids

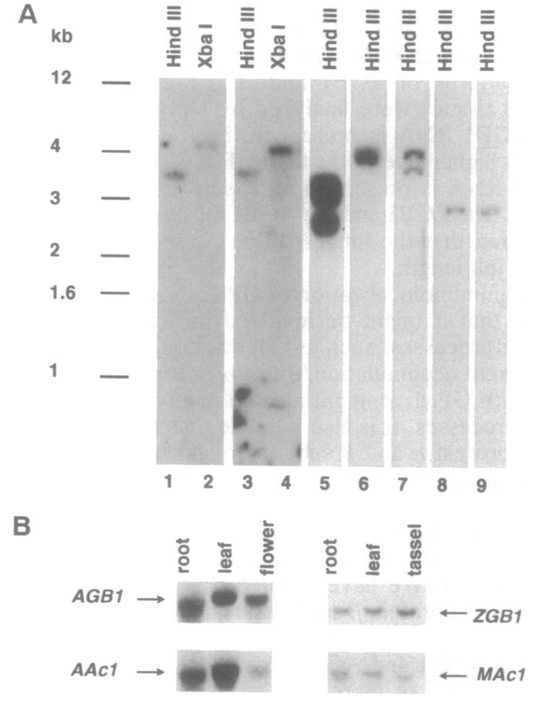

FIG. 5. (*A*) Southern blot analysis. Lanes: 1–4, *Arabidopsis* genomic DNA probed with a portion of AGB1 cDNA (see Fig. 2) and washed at high stringency (lanes 1 and 2) or low stringency (lanes 3 and 4); 5–7, genomic DNA of broccoli, bean, and carrot (respectively from the three families *Cruciferae*, *Leguminosae*, and *Umbelliferae*) probed with a portion of AGB1 cDNA and washed at low stringency; 8 and 9, maize genomic DNA probed with a portion of ZGB1 cDNA (see Fig. 1) and washed at high stringency (lane 8) and low stringency (lane 9). Each lane contains ≈5 μg of DNA. (*B*) Northern blot analysis of total *Arabidopsis* RNA (*Left*) extracted from tissues of 2- to 5-week-old plants and of total maize RNA (*Right*) extracted from tissues of 6-day-old seedlings (root and leaf) or from mature plants (tassel) and probed with either the full-length cDNA from AGB1 or ZGB1 and with an *Arabidopsis* actin cDNA (AAcl; ref. 44) or a maize actin cDNA (MAcl; ref. 45). Approximately 5 μg of RNA was loaded in each lane.

of two related diploids), two bands were observed, suggesting that both of the expected homologues were detected. As indicated by both library screening and DNA blots, the hybridization between homologues from several plants serves as a control for the absence of hybridization of additional sequences in maize and *Arabidopsis* genomes. The fact that both maize and *Arabidopsis* contain no other sequences that are very similar to these G protein β subunit genes suggests that these genes have a unique function. Furthermore, the fact that the G protein β subunit gene *AGB1* is conserved in flowering plants suggests that it plays one or more important roles in plant signaling.

However, our results do not rule out the possibility that other G protein β subunits are present in flowering plants. Nevertheless, they suggest that in plants, if other β subunits are present, they are only distantly related to ZGβ1/AGβ1, as in *Drosophila* where there are two very different β subunits. Biochemical studies have shown that in plants various cellular functions may be mediated by GTP-binding proteins, suggesting that more than one G protein is involved in the transmission of signals in relation to these cellular functions (8). Furthermore, several studies using cholera or pertussis toxins have produced evidence for the presence of G proteins susceptible to either one or both of these toxins (9–12). On the other hand, the only G protein α subunit whose gene has been cloned thus far lacks the C-terminal cysteine for ADP-ribosylation by pertussis toxin (13). So in plants, there may be other G protein α subunits as well as G protein β subunits encoded by genes that are very different from the cloned genes (*GPA1*, *TGA1*, *ZGB1*, and *AGB1*).

Expression of *ZGB1* and *AGB1*. To determine the expression patterns of ZGB1 and AGB1 cDNAs, RNAs from several tissues were analyzed by Northern hybridizations. The ZGB1 RNA is present in the root, the leaf, and at a slightly higher level in the tassel (Fig. 5*B*). In *Arabidopsis*, the AGB1 RNA is present in all tissues tested: root, leaf, and flower. The ZGB1 and the AGB1 messages are about 1.7 kb, indicating that the longest cDNA clones are full length or nearly full length.

The immunolocalization of GPα1 in *Arabidopsis* has suggested that it might be involved in a number of different cellular processes, such as cell division, cell differentiation, or nutrient accumulation/transport (46). If AGβ1 is associated with GPα1, it might also be involved in some of these same processes. It is also possible that within the same cell, the G protein α and β subunits might be involved in transducing different signals to different effectors, resulting in the activation of different cascades of events and cellular responses and that in other cell types, this same G protein might be involved with yet other receptors and effectors.

Conclusion. We have isolated ZGB1 and AGB1 cDNAs coding for G protein β subunits from maize and *Arabidopsis*, respectively. The amino acid sequences of the predicted proteins, ZGβ1 and AGβ1, are 76% identical to each other and show 41% or more identity to mammalian G protein β subunits. They contain seven repeats of the WD-40 motif that is found in all of the known G protein β subunits. Based on these results, we conclude that ZGβ1 and AGβ1 are G protein β-subunit homologues in maize and *Arabidopsis*, respectively. These results provide a further demonstration that a heterotrimeric G protein signaling pathway exists in plants and also point out structural differences between animal and plant G protein β subunits. The deduced proteins represent an additional type of G protein β subunits that are conserved among flowering plants and that are expressed in several organs. These genes provide new molecular tools to study G protein functions in plants.

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