Plant Physiol. (1995) 108: 1315–1316

Plant Gene Register

Cloning and Sequencing Analysis of a Full-Length cDNA Encoding a G Protein α Subunit, *SGA1*, from Soybean[1]

Woe Yeon Kim, Na Eun Cheong, Dong Chul Lee, Dae Yeop Je, Jeong Dong Bahk, Moo Je Cho, and Sang Yeol Lee*

Plant Molecular Biology and Biotechnology Research Center, Department of Biochemistry, College of Natural Sciences, Gyeongsang National University, Chinju, 660–701, Korea

The superfamily of G proteins consists of several families including translational factors, tubulins, and *ras*-related small molecular weight and heterotrimeric G proteins (Kaziro et al., 1991). The membrane-bound signal-transducing G proteins are heterotrimers composed of α subunits (39–52 kD), β subunits (35–36 kD), and γ subunits (7–10 kD) (Gilman, 1987). Different heterotrimeric G proteins have distinct α subunits that contain a high-affinity-binding site for guanine nucleotides and exhibit GTPase activity. In response to interaction with specific receptors, the α subunit of each G protein binds GTP and dissociates from the complex of the βγ subunits. The free α subunit is then able to interact with a specific effector to convert its activity from inactive precursor to active form. After hydrolysis of bound GTP by its intrinsic GTPase activity, the α subunit makes a conformational change that favors re-association of α with βγ (Conklin and Bourne, 1993). Because the specificity of the interaction of each heterotrimeric G protein with its effector resides in the α subunit, extensive studies have been carried out for the specific function of various G protein α subunits in nonplant organisms (Simon et al., 1991). Several reports have also been presented for the possible function of plant G protein α subunits on light and auxin signal transduction, phytochrome-regulated gene expression, and ion-channel current regulations (Fairley-Grenot and Assmann, 1991; Romero and Lam, 1993). Nevertheless, the precise roles and characteristics for the α subunits of plant G proteins are largely unknown, and only a few cDNAs encoding α subunits of plant G proteins have been cloned (Terryn et al., 1993).

Here we describe the identification of a soybean cDNA that encodes a heterotrimeric G protein α subunit by screening a soybean (*Glycine max* L.) λZAPII cDNA library (Table I). The clone of a G protein α subunit of *Arabidopsis thaliana* was used as a probe. From the 12 positive clones of 1×10^6 recombinant phage plaques, the longest cDNA insert was selected and sequenced. It contained a full-length cDNA encoding a soybean G protein α subunit, which was designated *SGA1*. The soybean cDNA clone, *SGA1*, is 1624 bp and has an open reading frame of 1155 bp encoding 385 amino acids, 135 bp of the 5′ untranslated region, and 334 bp of the 3′ untranslated region. The amino acid sequence comparison of SGA1 with the entries in the EMBL and SwissProt sequence data base revealed that the deduced amino acid sequence of SGA1 was most similar, 82.1 and 81.3% identical, to the plant G protein α subunits of *A. thaliana* (Ma et al., 1990) and tomato (Ma et al., 1991), respectively. However, SGA1 showed amino acid sequence identity between 30 and 40% with other representative G protein α subunits of human, rat, yeast, and *Drosophila* (Bourne et al., 1991; Kaziro et al., 1991). Despite its low

Table I. *Characteristics of a soybean cDNA encoding a G protein α subunit*

Organism:	Soybean (*Glycine max* L.).
Function:	*SGA1* gene encodes the α subunit of heterotrimeric GTP-binding protein. Putative GTPase.
Techniques:	Automatic double-stranded plasmid sequencing; restriction fragment subcloning and dideoxy sequencing of both strands.
Clone Type:	cDNA, full length, *SGA1*.
Source:	cDNA library in λZAPII vector constructed from poly(A^+) RNA of soybean seedling.
Method of Identification:	Isolation from a cDNA library constructed from soybean seedling mRNA. Library was screened with a probe of *GPA1*, the cDNA clone of an α subunit of heterotrimeric GTP-binding protein in *A. thaliana*.
Structural Features of the Protein:	Deduced amino acid sequence of 385 amino acids with a molecular mass of 44,949 D and a calculated pI of 5.36.
Expression Characteristics:	A transcript of approximately 1.7 kb was detected by northern blot analysis of poly(A^+)-enriched RNA; low level of mRNA is expressed in elongating, apical, and hypocotyl regions of soybean seedling.
Gene Copy Number:	Two strong and a few weak hybridizing DNA fragments can be seen in soybean genome cleaved by several restriction enzymes.
Antibodies:	Not available.

[1] This research was supported by a grant from the Plant Molecular Biology and Biotechnology Research Center (PMBBRC) in Korea.

* Corresponding author; e-mail sylee@nongae.gsnu.ac.kr; fax 82-591-759-9363.

amino acid sequence homology to nonplant G protein α subunits, SGA1 contains typical structural sequence motifs that are the characteristic features of all G protein α subunits (Bourne et al., 1991). The highly conserved amino acids reported to be involved in the GTPase activity and the binding region for the guanine ring of GTP were contained in the deduced amino acid sequence of SGA1. In addition to these conserved domains, several amino acid residues that are important for biological activity of G protein α subunits were also preserved; Arg^{191}, the putative ADP ribosylation site by cholera toxin, and Gly^2 (in the $M^1G^2X_3S^6$ domain at the amino terminus), the presumptive myristoylation site that may play an important role in enhancing binding of α with $\beta\gamma$ subunits, were also conserved in SGA1. However, SGA1 lacks the Cys residue, the fourth amino acid from the COOH-terminal end, which is the target site for pertussis toxin-mediated ADP ribosylation (Simon et al., 1991).

To investigate the expression level of *SGA1*, mRNA was isolated from different tissues of 5-d-old soybean seedling and used for northern blot analysis. A transcript of approximately 1.7 kb mRNA hybridized to the cDNA insert of *SGA1* probe. Even though the mRNA level of *SGA1* was low, it showed a tissue-specific distribution. The *SGA1* mRNA was predominantly in the elongating region of a soybean seedling, with low levels detected in apical and hypocotyl tissues. When Southern blot analysis was carried out by using the coding region of *SGA1* as a probe, two strong and a few weak hybridizing bands were observed. This result suggested that the α subunits of soybean G protein constituted a multigene family in soybean. However, it is not clear whether weak hybridizing bands represented additional genes of soybean G protein α subunits or other closely related homologs.

ACKNOWLEDGMENT

We thank Dr. E.M. Meyerowitz of the California Institute of Technology for generously providing the *GPA1* cDNA clone of *A. thaliana*.

Received December 20, 1994; accepted January 26, 1995.
Copyright Clearance Center: 0032-0889/95/108/1315/02.
The GenBank accession number for the sequence reported in this article is L27418.

LITERATURE CITED

Bourne HR, Sanders DA, McCormick F (1991) The GTPase superfamily; conserved structure and molecular mechanism. Nature **349:** 117–127

Conklin BR, Bourne HR (1993) Structural elements of Gα subunits that interact with G$\beta\gamma$, receptors, and effectors. Cell **73:** 631–641

Fairley-Grenot K, Assmann SM (1991) Evidence for G-protein regulation of inward K^+ channel current in guard cells of fava bean. Plant Cell **3:** 1037–1044

Gilman AG (1987) G proteins; transducers of receptor-generated signals. Annu Rev Biochem **56:** 615–649

Kaziro Y, Itoh H, Kozasa T, Nakafuku M, Satoh T (1991) Structure and function of signal transducing GTP-binding proteins. Annu Rev Biochem **60:** 349–400

Ma H, Yanofsky MF, Huang H (1991) Isolation and sequence analysis of *TGA1* cDNAs encoding a tomato G protein α subunit. Gene **107:** 189–195

Ma H, Yanofsky MF, Meyerowitz EM (1990) Molecular cloning and characterization of *GPA1*, a G protein α subunit gene from *Arabidopsis thaliana*. Proc Natl Acad Sci USA **87:** 3821–3825

Romero LC, Lam E (1993) Guanine nucleotide binding protein involvement in early steps of phytochrome regulated gene expression. Proc Natl Acad Sci USA **90:** 1465–1469

Simon MI, Strathmann MP, Gautam N (1991) Diversity of G proteins in signal transduction. Science **252:** 802–808

Terryn N, Van Montagu M, Inze D (1993) GTP binding proteins in plants. Plant Mol Biol **22:** 143–152