Heterotrimeric G proteins are mediators that transmit the external signals via receptor molecules to effector molecules. The G proteins consist of three different subunits: α, β, and γ subunits. The cDNAs or genes for all the α, β, and γ subunits have been isolated from many plant species, which has contributed to great progress in the study of the structure and function of the G proteins in plants. In addition, rice plants lacking the α subunit were generated by the antisense method and a rice mutant, Daikoku d1, was found to have mutation in the α-subunit gene. Both plants show abnormal morphology such as dwarfism, dark green leaf, and small round seed. The findings revealed that the G proteins are functional molecules regulating some body plans in plants. There is evidence that the plant G proteins participate at least in signaling of gibberellin at low concentrations. In this review, we summarize the currently known information on the structure of plant heterotrimeric G proteins and discuss the possible functions of the G proteins in plants.

Key words: Gibberellin — Heterotrimeric G protein — Signal transduction.

Subunits of heterotrimeric G proteins in plants

The cDNAs or genes for the α subunits of plant heterotrimeric G proteins have been isolated on the basis of sequence homology to mammalian subunits from Arabidopsis (Ma et al. 1991), rice (Ishikawa et al. 1995, Seo et al. 1995), oat (Jones et al. 1998), tomato (Ma et al. 1991), soybean (Gotor et al. 1996, Kim et al. 1995), pea (Marsh and Kaufman 1999), Nicotiana plumbaginifolia (Kaydmov et al. 2000), spinach (Perroud et al. 2000), and Lotus japonicus (Poul sen et al. 1994). The predicted amino acid sequences of all proteins encoded by the cDNAs and genes contain sequences similar to those for the functional domains such as a myristylation site, four GTP-binding sites, a cholera toxin-binding site, three effector-binding sites and a receptor-binding site, which have been identified in the mammalian α subunits (Kaziro et al. 1991). The sequences for some effector-binding sites are highly conserved among plants, mammals and yeast, suggesting that some effectors in the organisms may be very similar to one another. In contrast, the sequences for the receptor-binding regions of the organisms exhibit much lower grades of identity, suggesting that receptor molecules in the three species of organisms may be quite different from one another. The α subunits are known to be the regulatory subunits for GTP–GDP exchange in mammalian heterotrimeric G proteins. Recombinant protein for the α subunits of plant origin of cDNAs display GTPase and GTPγS-binding activities (Iwasaki et al. 1997, Seo et al. 1997, Wise et al. 1997), indicating that the cDNAs encode the true α subunits with similar functions to those of the mammalian subunits.

The cDNAs or genes proposed to encode the β subunits have also been isolated from many plant species including Arabidopsis (Weiss et al. 1994, rice (Ishikawa et al. 1996), oat (Jones et al. 1998), maize (Weiss et al. 1994), and tobacco (Kaydmov et al. 2000, Kusnetsov and Oelmuller 1996). The proteins have two particular structures, an N-terminal sequence and seven repetitive WD-40 repeats observed in the mammalian β subunits. The N-terminal part is considered to interact with the α subunit through the formation of a coiled-coil structure. The WD-40 repeats have been proposed to form a specific motif performing protein–protein interactions with effector molecules.

A cDNA proposed to encode the γ subunit was very recently isolated from Arabidopsis (Mason and Botella 2000). The predicted amino acid sequences of the putative γ subunit show characteristics typical of the mammalian γ subunits; namely there are two putative functional domains, an N-terminal α-helix region capable of forming a coiled-coil interaction with the β subunits and a C-terminal CAAX box to direct isoprenyl modification.

Each mammalian genome contains large multigene families for the subunits of heterotrimeric G proteins; the families for the α, β, and γ subunits consist of more than 20, 5, and 11 genes, respectively (Offermanns 2000). Interestingly, each plant genome contains only one or two genes for each subunit. Thus, plant heterotrimeric G proteins may be involved in only a limited number of signaling pathways.

Is the G protein actually a heterotrimer?

In 2000, the plant γ subunit was shown to interact with the plant β subunit by two different approaches, the two-hybrid method and in vitro translation experiment (Mason and Botella 2000). However, no evidence has been presented for the inter-
action between the α subunit and the βγ dimer in plants. Since plant heterotrimeric G proteins have not yet been purified, there is no proof that a heterotrimeric G protein composed of the α, β, and γ subunits is really present in plant cells, and so further investigations are necessary to elucidate the structure of plant heterotrimeric G proteins.

How many types of heterotrimeric G proteins are present in higher plants?

Plant heterotrimeric G proteins have been proposed to mediate quite different signals, such as plant hormones (Hooley 1999, Lovegrove and Hooley 2000), elicitor (Blumward et al. 1998) and light (Barbier-Brygoo et al. 1997, Barnes et al. 1997). Therefore, attention should be directed to the question of whether a single species of heterotrimeric G protein receives these different signals via receptors or different types of heterotrimeric G proteins independently recognize different signals. Both the α subunits and βγ dimers are known to be functional and to separately regulate effector molecules in mammals. Available data reported thus far suggest the presence of a single gene for either the α subunit or the β subunit in each genome for Arabidopsis and rice, and so it may be reasonable to postulate that each plant species fundamentally contains a single molecular species of heterotrimeric G protein. This raises another important question of whether it is the α subunit or the βγ dimer that functions in the regulation of a given effector in plants.

Another possibility is that some higher plants contain plural molecular species of heterotrimeric G protein. Two cDNA species for the α subunits have been isolated in pea (Marsh and Kaufman 1999) and soybean (Gotor et al. 1996, Kim et al. 1995). No significant differences have been observed in both effector- and receptor-binding sites between the two species of α subunits found in pea or soybean, but the amino acid sequences of the N-terminal parts of the two species are not similar to each other. Since the N-terminal region is considered to be a recognition site for the βγ dimer, there may be two types of β subunits, which interact separately with the two species of α subunits in these plant species. The two types of pea α subunits share less than 88% identity in the amino acid sequence, and there are differences in the tissue-specific expression pattern of transcripts between the two subunits.

Two cDNA species have also been isolated for the β subunits in oat (Jones et al. 1998) and Nicotiana plumbaginifolia Viv (Kaydmov et al. 2000), but the amino acid sequence of only one of the two β subunit species has been reported for both plants. In N. plumbaginifolia Viv, there is no correlation in the expression pattern between the α subunit and one of the two β subunits. This result suggests that the α subunit may not be a counterpart of the β subunit, and an additional α subunit may interact with the β subunit.

Considering the results described above, there may be several species of the α and β subunits in some plant species. Other types of α or β subunits may not have been detectable because of much lower levels of sequence homology. Our search did not reveal other sequences highly homologous to the α subunits of Arabidopsis (GPA1) and rice (RGA1) in the Arabidopsis genome sequence.

Where are the heterotrimeric G proteins localized in a plant cell?

The α and β subunits have been shown to be localized in the plasma membrane fractions in Arabidopsis (Weiss et al. 1997), rice (Iwasaki et al. 1997) and tobacco (Peskan and Oelmuller 2000). This is reasonable because heterotrimeric G proteins must interact with receptor molecules present in the plasma membrane. In addition to the plasma membrane fractions, the α subunit was also found in the endoplasmic membrane fraction from Arabidopsis (Weiss et al. 1997), and the β subunit, in a purified nuclear fraction from tobacco (Peskan and Oelmuller 2000). In mammals, some heterotrimeric G proteins regulate the vesicle trafficking (Lang 1999) and others are associated with the nuclear membrane and the cytoskeleton (Willard and Crouch 2000). In rice, a heterotrimeric G protein regulates the biosynthesis of α-amylase in seeds (Ueguchi-Tanaka et al. 2000). Because the α-amylase is a secretory protein, plant heterotrimeric G proteins may also regulate the vesicle trafficking as do the mammalian G proteins.

Molecules related to heterotrimeric G proteins in plants

Two clones for a developmentally regulated G protein (Etheridge et al. 1999) and an extra-large GTP-binding protein (Lee and Assmann 1999) have been isolated from Arabidopsis as new classes of G proteins. Both proteins have GTP-binding motifs, and thus are members of the G-protein superfamily. However, they show little sequence identity with the α subunits of heterotrimeric G proteins. The extra-large GTP-binding protein may bind the β subunit of heterotrimeric G protein, because it has a putative binding region to the β subunit. The functions of these two G proteins, however, remain unknown.

Mammalian G protein-coupled receptors are known to have typical domains, possess seven transmembrane spanning domains, and organize a large protein family (Bockaert and Pin 1999). In the plant kingdom, more than 20 proteins with similar structures have been reported (Devoto et al. 1999, Hansen et al. 1997, Josefsson and Rask 1997, Plakidou-Dymock et al. 1998), but their functions remain unknown.

Biochemical functions of the subunits of heterotrimeric G proteins in plants

The α subunit of a heterotrimeric G protein was shown by in vitro experiments to activate a plasma membrane Ca\(^{2+}\) channel in tomato (Aharon et al. 1998). Regulation of the Ca\(^{2+}\) movement by the G protein has been proposed to be involved in elicitor signaling. This offered direct proof for a function of the α subunit; other studies on the functions of the plant G protein have presented only suggestive data, such as the effects of activators and inhibitors of the mammalian G proteins on the signal transduction in plant cells.
Involvement of the $\alpha$ subunit of rice heterotrimeric $G$ protein (RGA1) in normal development of internodes and seeds

Suppression of the mRNA for the $\alpha$ subunit of rice heterotrimeric $G$ protein (RGA1) through antisense technology caused morphological changes in rice plant (Fujisawa et al. 1999). The transformants lacking in mRNA for RGA1 exhibit abnormal morphology including dwarfism (due to shortening of internodes), small round seed and dark green leaf. This led to the discovery that the rice dwarf mutant, Daikoku $d1$, a traditionally famous mutant, has mutation in the RGA1 gene (Ashikari et al. 1999, Fujisawa et al. 1999). Daikoku $d1$ exhibits abnormal morphology very similar to that of the transformants lacking RGA1 mRNA. Phenotypes of normal cultivar and Daikoku $d1$ are shown in Fig. 1. The sequence analysis of the $\alpha$-subunit genes of more than 10 Daikoku $d1$ alleles shows that all the alleles have different types of mutations in the open reading frame of the RGA1 gene, such as amber mutation, ochre mutation, insertion and deletion. The antibody against the recombinant RGA1 recognized a 45-kDa polypeptide which was localized in the plasma membrane from normal cultivars, but the immunoreactive polypeptide was not detected in the membrane from all Daikoku $d1$ alleles (Fujisawa, unpublished data). Since Daikoku $d1$ could be complemented by introduction of endogenous RGA1 (Ashikari et al. 1999), it is evident that the abnormal phenotypes of Daikoku $d1$ are due to loss of the function of “a single RGA1 gene”. It would be of great interest to generate dicotyledonous knocking out of the $\alpha$-subunit gene(s) and to observe what types of phenotypes the mutants exhibit.

As no significant difference was observed in the cell length between the internodes of normal cultivar rice and Daikoku $d1$, the number of cells in each internode may be reduced in Daikoku $d1$. It remains to be investigated whether or not the heterotrimeric G protein regulates the velocity of cell division. The supposition that the higher plant G proteins may participate in cell growth and differentiation has been done on the basis of the pattern of expression of the $\alpha$ subunit in Arabidopsis (Huang et al. 1994).

Daikoku $d1$ has a partial defect in gibberellin signaling

Daikoku $d1$ has been suggested to be a gibberellin insensitive mutant (Mitsunaga et al. 1994). Genes of $\alpha$-amylase and GAmyb, a transcription factor to the $\alpha$-amylase gene, are known to be activated by gibberellin, and thus the genes are typical molecular markers for gibberellin (Lovegrove and Hooley 2000, Mitsui and Itoh 1997). When gibberellin (GA$_3$) at concentrations below $10^{-7}$ M was applied to embryo-less half seeds of rice, the mRNAs for GAmyb and $\alpha$-amylase were induced in normal cultivar seeds, but not in Daikoku $d1$ (Ueguchi-Tanaka et al. 2000). The mRNAs for GAmyb and $\alpha$-amylase in Daikoku $d1$ seeds increased as did those in normal cultivars, when GA$_3$ at concentrations higher than $10^{-7}$ M was applied to the seeds. Similarly, elongation of the second leaf sheath was enhanced for normal cultivars, but not for Daikoku $d1$, when applied with GA$_3$ at concentrations below $10^{-7}$ M, and the enhancement was observed with Daikoku $d1$ as well as with normal cultivars, when GA$_3$ was used at concentrations higher than $10^{-7}$ M. These results suggest that there may be at least two different pathways in gibberellin signaling and that heterotrimeric G proteins appear to be involved in a signaling pathway of GA$_3$, at low concentrations, but not at high concentrations (Fig. 2B).

A gibberellin-related recessive mutant, slender rice (sln), responds constitutively to gibberellin (Ikeda et al. personal communication). This mutant has a defect in a gene with a homologous function to that of GAI (Peng et al. 1997) or RGA (Silverstone et al. 1998) gene, which acts as a negative regulator in gibberellin signaling in Arabidopsis. Crossing experiments with Daikoku $d1$ and sln show that sln is epistatic to Daikoku $d1$, implying that the heterotrimeric G protein is a component of gibberellin signaling. We have found no differences in physiological responses to other phytohormones tested thus far between normal cultivars and Daikoku $d1$.

Possible signaling pathways involving plant heterotrimeric G proteins

Mastoparan, GTP$_\gamma$ S, and cholera toxin are known to acti-
Heterotrimeric G proteins in plants

vate mammalian heterotrimeric G proteins, and GDPβS and pertussis toxin, to repress them (Kaziro et al. 1991). However, as these activators and inhibitors are not specific regulators for the G proteins, the experimental results with these chemicals should very carefully be analyzed. Most of the functions of plant heterotrimeric G proteins have been proposed from studies with such chemicals (Fig. 2A). Consequently, we suggest that the proposed functions should be re-examined by other approaches. In addition, some researchers have reported that some plant signaling pathways are sensitive to pertussis toxin in which heterotrimeric G proteins may be involved. However, all plant α subunits reported have no target site for pertussis toxin, which is known to be a cysteine residue present near the C-terminal in mammalian α subunits. What needs to be investigated is whether there is a new species of α subunit modified by pertussis toxin in plants, or the plant α subunits reported can be modified by pertussis toxin at an unassigned motif other than the target site in the mammalian subunits.

Plant hormone signaling

*Gibberellin signaling*—Mastoparan 7, an analog of mastoparan, was found to induce α-amylase synthesis in wild oat aleurone (Jones et al. 1998). Since its action mimics gibberellin, a heterotrimeric G protein was proposed to be involved in the gibberellin signaling. The optimal concentration of mastoparan 7 in the physiological experiments was 1–4 μM and mastoparan 7 at above 10 μM caused artificial cell death. As discussed in Section 4, studies with Daikoku dl revealed that the rice heterotrimeric G protein participates in a signaling pathway of gibberellin at low concentrations but not at high ones.

*Abscisic acid signaling*—GTPγS stimulates barley aleurone phospholipase D in the same manner as does abscisic acid (Ritchie and Gilroy 2000). The activity of phospholipase D is blocked by GDPβS and pertussis toxin. Since the activation of phospholipase D by abscisic acid has also been observed in guard cells (Jacob et al. 1999), the activation of phospholipase...
D by abscisic acid may be a general event in plants. Involvement of plant heterotrimeric G proteins in phospholipase D signaling has been proposed, on the basis of stimulation of the enzyme by mastoparan (Munnik et al. 1995). In addition, a heterotrimeric G protein was suggested to be involved in a lipid signaling pathway leading to root hair formation (Hartog et al. 2001). In this case, the external signal for the activation of phospholipase D is *Rhizobium*-secreted nodulation factors and not abscisic acid.

The inward K⁺ channel in guard cells seems to be regulated by a heterotrimeric G protein (Fairley-Grenot and Assmann 1991, Kelly et al. 1995, Wu and Assmann 1994). Regulators of the G protein appear to inhibit inward K⁺ channel in the cells and to produce effects similar to those of abscisic acid. In this case, both cholera toxin, an activator of the mammalian G protein, and pertussis toxin, an inhibitor of the G protein, inhibit the inward K⁺ channel in similar manners (Fairley-Grenot and Assmann 1991); this would be interpreted as suggesting that a new G protein is modified by either toxin in order to participate in the regulation of this channel.

**Auxin signaling**—Mastoparan stimulates phospholipase A2 in a similar manner to that for 2,4-dichlorophenoxyacetic acid (Scherer 1992).

**Cytokinin signaling**—The antisense suppression of the expression of a G protein-coupled receptor (GCR1) in Arabidopsis leads to a phenotype like that exerting action of cytokinin signaling (Hooley 1999).

**Light signaling**

In aurea cells of tomato, GTPγS and cholera toxin stimulate the expression of chlorophyll a,b-binding protein (cab), chalcone synthase (chs), and ferredoxin-NADP⁺ oxidoreductase (fnr) genes in the same manner as light signal works, and Ca²⁺ and cGMP stimulate the transcription of *cab* and *chs* genes, respectively (Bowler et al. 1994a, Bowler et al. 1994b). In mammals, both Ca²⁺ and cGMP are known to be messengers regulated by heterotrimeric G proteins. In soybean cells, cholera and pertussis toxins activate the expression of *cab* gene even in the dark (Romero and Lam 1993). Observations of both cholera and pertussis toxins having the same physiological effects on downstream events, have been reported for the inward K⁺ channel as stated before. In the plasma membrane of the apical bud of etiolated peas, a blue light stimulates GTP-binding activity (Warphbeha et al. 1991). The putative α subunit of a pea heterotrimeric G protein is ADP-ribosylated by blue light, and the ADP-ribosylation is inhibited by pertussis toxin. These findings support the proposal of participation of a heterotrimeric G protein in light signaling in plants. Similarly, a potential heterotrimeric G protein may have been proposed as a transducer in light signaling in green alga (Calenberg et al. 1998).

**Pathogen signaling**

Mastoparan increases inositol 1,4,5-triphosphate in soybean cells as observed with a polygalacturonic acid elicitor (Legendre et al. 1993), suggesting that a heterotrimeric G protein activates phospholipase C after receiving a signal for pathogen infection. Mastoparan also stimulates H₂O₂ production (Legendre et al. 1992). In addition, cholera toxin activates signaling pathways for pathogen resistance in tobacco transformants (Beffa et al. 1995). These finding suggest that heterotrimeric G proteins may work as mediators in pathogen-mediated signaling in plants.

**Others**

GTPγS and cholera toxin stimulate the germination and tube elongation of lily pollen (Ma et al. 1999), whereas GDPβS and pertussis toxin inhibit the phenomena. Although mastoparan was found to have no effect on the elongation of pollen tubes in *Papaver rhoeas* (Franklin-Tong et al. 1996), the concentration of mastoparan used in this work seemed to be so high that it might cause cell damage.

**Conclusion**

At present, only limited pieces of evidence have been presented concerning the structure and function of plant heterotrimeric G proteins, although much suggestive information is available. The cDNAs and genes for the α, β, and γ subunits of the plant G proteins have been isolated through probing with sequence homology to mammalian types. Studies with recombinant proteins have revealed the characteristics of plant α subunits, and some information is available on the interaction among the subunits. There is, however, no conclusive evidence for the actual existence of a heterotrimer of the α, β, and γ subunits in plants, and no data have been presented for the dissociation of the heterotrimer into the α subunit and βγ dimer to transmit the received signals to effectors. Furthermore, the number of heterotrimeric G proteins in each plant species should be re-examined although only one molecular species of the G proteins has been suggested to be present in Arabidopsis.

It is now evident that plant heterotrimeric G proteins are involved in the normal development of some organs. Rice plants lacking in the α subunit, for example, Daikoku *d1*, show abnormal morphology including dwarfism. Undoubtedly, the plant G proteins participate in the signaling of gibberellin at low, but not high, concentrations. Proposals have been made that the G proteins may be concerned in many other signaling pathways in plants, but remain to be investigated to present conclusive evidence. Also, the receptors and effectors for the plant G proteins have not yet been conclusively identified.

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