Two *O*-Linked *N*-Acetylglucosamine Transferase Genes of *Arabidopsis thaliana* L. Heynh. Have Overlapping Functions Necessary for Gamete and Seed Development

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ABSTRACT

The Arabidopsis SECRET AGENT (SEC) and SPINDLY (SPY) proteins are similar to animal *O*-linked *N*-acetylglucosamine transferases (OGTs). OGTs catalyze the transfer of *N*-acetylglucosamine (GlcNAc) from UDP-GlcNAc to Ser/Thr residues of proteins. In animals, *O*-GlcNAcylation has been shown to affect protein activity, stability, and/or localization. SEC protein expressed in *Escherichia coli* had autocatalytic OGT activity. To determine the function of *SEC* in plants, two tDNA insertional mutants were identified and analyzed. Although *sec* mutant plants did not exhibit obvious phenotypes, *sec* and *spy* mutations had a synthetic lethal interaction. This lethality was incompletely penetrant in gametes and completely penetrant postfertilization. The rate of both female and male *sec spy* gamete transmission was higher in plants heterozygous for both mutations than in plants heterozygous for *sec* and homozygous for *spy*. Double-mutant embryos aborted at various stages of development and no double-mutant seedlings were obtained. These results indicate that OGT activity is required during gametogenesis and embryogenesis with lethality occurring when parentally derived SEC, SPY, and/or *O*-GlcNAcylated proteins become limiting.

THE Arabidopsis *SPINDLY* (*SPY*) gene product is an important component of the gibberellin signaling pathway (Jacobsen and Olszewski 1993; Jacobsen *et al.* 1996; Thornton *et al.* 1999a; Sun 2000). Gibberellins (GA) are a family of dicyclic terpenoid plant hormones that affect many aspects of plant growth and development including germination, hypocotyl elongation, leaf greening, elongation growth, flowering time, and seed filling (Hedden and Phillips 2000; Lovegrove and Hooley 2000; Sun 2000; Yamaguchi and Kamiya 2000). Mutations in *SPY* suppress the effects of GA deficiency, whether this deficiency is caused genetically or by chemical inhibitors of GA biosynthesis (Jacobsen and Olszewski 1993; Wilson and Somerville 1995; Peng *et al.* 1997; Silverstone *et al.* 1997), without restoring GA biosynthesis (Peng *et al.* 1999; Silverstone *et al.* 2001). Because *spy* mutations can suppress GA deficiency and because all known *SPY* mutations are recessive, *SPY* negatively regulates GA signaling (Jacobsen and Olszewski 1993; Jacobsen *et al.* 1996; Swain and Olszewski 1996; Thornton *et al.* 1999a; Sun 2000). Additional evidence that *SPY* acts as a negative regulator of GA signaling has come from the analysis of double mutants between *spy* and other GA signaling mutations (Wilson and Somerville 1995; Jacobsen *et al.* 1996; Peng *et al.* 1997; Silverstone *et al.* 1997; Peng *et al.* 1999) and from *SPY* overexpression studies (Robertson *et al.* 1998; Izhaki *et al.* 2001; Swain *et al.* 2001). However, *spy* mutations do not completely suppress mutations in GA biosynthesis genes and *spy* mutant plants are somewhat responsive to exogenously applied GA (Jacobsen and Olszewski 1993; Silverstone *et al.* 1997; Vivian-Smith and Koltunow 1999). Therefore, a portion of GA signaling may act through a route independent of *SPY*.

There is also evidence that *SPY* has roles beyond its role in GA signaling. Swain *et al.* (2001) found that *spy* mutants had subtle phenotypes that were not usually observed in GA-deficient mutants or plants treated with excessive GA, including reduced height, deviant phyllotaxy, and absence of leaf serration, and suggested *SPY* could also have a role in GA-independent developmental pathways.

The SPY protein has a significant level of similarity to animal *O*-linked *N*-acetylglucosamine transferases (OGTs; Kreppel *et al.* 1997; Lubas and Hanover 2000). OGTs are cytosolic and nuclear localized glycosyltransferases that transfer *N*-acetylglucosamine (GlcNAc) residues from UDP-GlcNAc to serines and threonines via an *O*-linkage (Hart 1997; Hanover 2001; Wells *et al.* 2001). The activity of the OGT enzyme itself is sensitive to the concentration of UDP-GlcNAc and may therefore serve as a sensor of carbohydrate level within cells (Kreppel and Hart 1999; Han *et al.* 2000). In mammalian cells, loss of OGT function is lethal (Shafi *et al.* 2000).

A large number of nuclear and cytosolic proteins are *O*-GlcNAc modified (Hart 1997; Wells *et al.* 2001).

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under accession no. AF441079.

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The *O*-GlcNAc modification of a protein has been shown to affect its stability (Han and Kudlow 1997), subcellular localization (Snow and Hart 1998), and/or interaction with other proteins (Roos *et al.* 1997). One mechanism by which *O*-GlcNAc addition can effect changes in protein activity is through the competition between *O*-GlcNAcylation and phosphorylation for modification of the same serine/threonine residues. Reciprocal phosphorylation/*O*-GlcNAcylation of specific amino acids has been demonstrated for the murine estrogen receptor β (Cheng *et al.* 2000; Cheng and Hart 2001), the carboxy-terminal domain of RNA polymerase II (Kelly *et al.* 1993; Comer and Hart 2001), and the transcription factor, c-*myc* (Chou *et al.* 1995), and the reciprocal modifications have been shown to differentially affect the activity of these proteins. Thus proteins can exist as the phosphorylated, hydroxylated, and *O*-GlcNAcylated forms, each with different properties. The presence of these three possible states creates an opportunity for regulation of protein activity through the regulated action of kinases, phosphatases, OGT, and/or the enzyme that removes *O*-GlcNAc residues, *O*-GlcNAse (Gao *et al.* 2001; Wells *et al.* 2002). It should also be noted that not all substrate proteins are regulated via reciprocal phosphorylation/*O*-GlcNAcylation, in some cases, *O*-GlcNAc may directly affect protein activity (Roos *et al.* 1997; Yang *et al.* 2001).

Preliminary evidence suggests that SPY has OGT activity *in vitro* (Thornton *et al.* 1999b; Thornton 2001) and that *spy* mutant plants have reduced levels of *O*-GlcNAcylated proteins (Thornton 2001). However, protein *O*-GlcNAcylation is reduced only in *spy* mutants, raising the possibility of additional OGT(s) in Arabidopsis.

In this article we describe the identification and cloning of the *SECRET AGENT* (*SEC*) gene of Arabidopsis. The predicted SEC protein resembles both SPY and animal OGT proteins. When expressed in *Escherichia coli*, the SEC protein was able to *O*-GlcNAc modify itself, a property exhibited by human OGT. To determine the functional role of *SEC*, tDNA insertional mutations were identified and their phenotypes were compared to wild type. Although *sec* insertional mutant lines did not exhibit obvious phenotypes, *sec* mutations exhibited synthetic lethality when in combination with mutations in *spy*. These observations indicate that *SEC* and *SPY* have overlapping functions and that OGT activity is essential in plants.

MATERIALS AND METHODS

Plant strains and growth conditions: All experiments were performed using *A. thaliana* (L.) Heynh. ecotype Columbia as wild type, the *spy-3* mutant in a Columbia background (Jacobsen and Olszewski 1993), and mutants isolated from tDNA-insertion line pools (described below). Plants were grown in a growth chamber with 16-hr light (22°) and 8-hr dark (20°) cycles supplied under a mixture of fluorescent and incandescent lights with an intensity of 85 μmol/m^2/sec.

Isolation of *SEC*-expressed sequence tags and genomic clones: Twelve expressed sequence tags (ESTs) encoding proteins with similarity to the tetratricopeptide repeat (TPR) domains of rat OGT (U76557; Kreppel *et al.* 1997) and SPY (U21320; Jacobsen *et al.* 1996) proteins were identified and ordered from the *Arabidopsis* Biological Resource Center (ABRC, Columbus, OH, http://www.arabidopsis.org). Three Arabidopsis (H76849, R64973, and W43557) EST clones were sequenced further on the basis of restriction mapping and cross hybridization. One Arabidopsis clone, H76849, predicted to encode a SPY/OGT-like protein (SEC), was used to obtain a cross-hybridizing genomic clone from an Arabidopsis library constructed in pOCA18 (Olszewski *et al.* 1988).

Assembly of full-length *SEC* cDNA clones: The H76849 EST clone was not full length and, therefore, the 5′ end of the SEC mRNA was obtained by 5′ random amplification of cDNA ends (5′ RACE). RNA was isolated from 1-week-old wild-type plants (Ausubel *et al.* 1992) and poly(A)-mRNA was purified from total RNA using the Poly(A)-Tract mRNA isolation kit (Promega, Madison, WI). For 5′ RACE, reverse transcription and second-strand synthesis were performed according to the 5′-RACE kit manufacturer's directions (Marathon cDNA amplification kit, CLONTECH, Palo Alto, CA) using 1.6 μg of poly(A)-RNA and the NS4 primer (TGATGAGGATCTGGA TTTTGTCTGG). The products of the second-strand synthesis reaction were ligated to the AP1 adapter and used as template for PCR (Expand high fidelity PCR system, Boehringer Mannheim, Indianapolis) with the AP1 and the NS4 primers. The product that hybridized to ^{32}P-labeled H76849 EST DNA was cloned into pCR2-TOPO (Invitrogen, Carlsbad, CA).

To assemble the full-length *SEC* cDNA, a *Not*I-*Stu*I 5′-RACE RT-PCR restriction fragment and a *Stu*I-*Not*I EST (H76849) restriction fragment were purified and cloned into the *Not*I site of pBluescript SK (Stratagene, La Jolla, CA; Ausubel *et al.* 1992).

DNA sequencing of cDNA and genomic clones: The full-length cDNA and a portion of the genomic clone corresponding to the gene were fully sequenced by primer walking at the University of Minnesota Advanced Genetics Analysis Center.

Expression of maltose-binding protein-SEC and -TPR: An *Xba*I fragment from the *SEC* cDNA clone was cloned into the *Xba*I site of pMAL 2c (New England Biolabs, Beverly, MA) to create the pMAL-SEC plasmid that encodes a maltose-binding protein (MBP)-SEC fusion protein. While the fusion protein does not contain the first 60 amino acids of SEC, it contains all of the TPRs and the full carboxy-terminal domain.

A second fusion-protein expression construct was made to serve as a negative control in experiments examining the OGT activity of MBP-SEC. This construct encodes a protein consisting of only the TPR portion of SEC fused to MBP. The pMBP-TPR plasmid was created by self-ligation of pMAL-SEC following digestion with *Stu*I and *Eco*RV.

E. coli (XL1Blue; Stratagene) containing pMBP-SEC or pMBP-TPR were grown at 22° to an OD_{600} of 0.6 and protein expression was induced with 0.3 mm isopropyl β-d-thiogalactopyranoside (Kroll *et al.* 1993) for 15 min, and then cells were pelleted and frozen. Bacteria were lysed using a French press and fusion proteins were purified by affinity chromatography on a sepharose-bound amylose column (New England Biolabs) according to manufacturer's directions. Purified proteins were resolved by SDS-PAGE (Laemmli 1970) and visualized by staining with Coomassie or transferred to PVDF membranes (Millipore, Bedford, MA).

Detection and characterization of protein GlcNAc modification: The terminal GlcNAc modifications of membrane-bound proteins were labeled with [^{3}H]galactose as described by Roquemore *et al.* (1994) and modified by Heese-Peck *et al.* (1995). For one or two 25-cm^2 membrane(s) the reaction

contained 12 μCi of 60 Ci/mmol [^{3}H]UDP-galactose (American Radiolabeled Chemicals, St. Louis) and 60 milliunits Gal β(1-4) galactosyl-transferase (GalT, Sigma) in 1 ml of GalT buffer (10 mM galactose, 10 mM HEPES, 5 mM $MnCl_2$, pH 7.4).

Glycosyl groups can be linked to proteins by either *O*- or *N*-linkage. To determine the linkage of the glycosyl groups to SEC, affinity-purified proteins were labeled and subjected to β-elimination or incubated with PNGase F, treatments that hydrolyze *O*- and *N*-linkages, respectively (Roquemore *et al.* 1994). Affinity-purified proteins were precipitated with 8 volumes of acetone, dried, and resuspended in 1% SDS. After [^{3}H]galactose labeling, proteins were separated from unincorporated label by gel filtration chromatography (Roquemore *et al.* 1994). One-milliliter fractions were collected and the radioactivity in each fraction was quantitated. The labeled protein fractions were pooled, acetone precipitated, and subjected to PNGase digestion or β-elimination (Roquemore *et al.* 1994). For PNGase digestion, 2500 units of PNGase F (New England Biolabs) was added to the precipitated sample, which was resuspended in the manufacturer's PNGase buffer and incubated at 37° for 3 hr and rechromatographed. For β-elimination, the precipitated sample was resuspended in 0.1 N NaOH at 37° for 24 hr, neutralized with chilled 4 M acetic acid, and rechromatographed.

Identifying *SEC* insertional alleles: *SEC* insertional mutants were obtained by screening DNA of pools of tDNA insertional lines by PCR as described by Winkler *et al.* (1998). The DNA and associated primers for two sets of tDNA-insertion lines (CD5-7, McKinney *et al.* 1995; CD6-7, Campisi *et al.* 1999) were obtained from the ABRC. In addition to the line-specific right and left border primers, *SEC*-specific primers, NS4 (see above) and NS1: ATAATGAGGTTCTTCGTATTGACCCAT were used in PCR reactions. The tDNA-insertion sites within the *SEC* gene were identified by sequencing. The *sec-1* mutant was isolated in a Wassilewskija background and backcrossed three times into Columbia; the *sec-2* mutant was in a Columbia background and was backcrossed twice.

Crossing experiments involving *sec-1* or *sec-2* and *spy-3*: Plants with the backcrossed *sec-1* or *sec-2* alleles were crossed to *spy-3* plants. A selection scheme was employed to identify + *spy/sec spy* plants (see RESULTS). For germination all seeds were surface sterilized (Robertson *et al.* 1998) and placed on MS germination medium in petri dishes. MS medium contained Murashige and Skoog salts (Life Technology GIBCO-BRL, Gaithersburg, MD; 1% sucrose, 0.05% 2-[*N*-morpholino] ethanesulfonic acid, and 0.6% phytoagar, pH 5.8). Seeds were imbibed on the MS plates at 4° for 3–5 days and then moved to 22° in constant light for 7–14 days. In some cases, selection for kanamycin resistance (Kan^R) or resistance to the GA biosynthesis inhibitor, paclobutrazol (PAC^R), was applied. For Kan selection, seeds were germinated on MS medium with 30 or 50 mg/liter of kanamycin (MS-Kan). For PAC selection, seeds were sown onto MS medium containing 35 mg/liter paclobutrazol (MS-PAC; Bonzai, UniRoyal Chemicals, Middlebury, CT).

Statistical comparisons of different populations: To compare whether plants from two different populations were segregating with different Kan^R:Kan^S ratios, chi-square contingency tests were performed (Whitehouse 1973). Two populations were not different when the deviations of the two populations were not greater than the deviations expected for the mean of a single population 99% of the time.

Genotyping of mutant and wild-type alleles: Allele-specific PCR or cleaved amplified polymorphic DNA (CAPs; Konieczny and Ausubel 1993) markers were used to differentiate between wild-type and mutant alleles. DNA was extracted from a single leaf using a DNA extraction protocol (Kidwell and Osborn 1992) scaled down to 100 μl and the resulting DNA was resuspended in 10 μl of 10 mM Tris-HCl, pH 8.0, 1 mM EDTA. PCR reactions used 0.25 units of Taq polymerase (Fisher Scientific, Hampton, NH), in the manufacturer's 1× buffer with 1 μl of DNA extraction, 0.25 μM primers, and 160 μM dNTPs in 15 μl. Alternatively, DNA was prepared and PCR amplified with the REDExtract-N-Amp (Sigma) kit. All reactions were heated to 94° for 2 min and then cycled for 35 times using a cycling program of 94° for 15 sec, 50° for 15 sec, 72° for 70 sec. For *sec-1*, left tDNA border (GATGCAATC GATATCAGCCAATTTTAG) and NS1R primers were used to generate a 400-bp fragment. For detection of the wild-type *SEC* allele corresponding to *sec-1*, GSP27 (AATGGGCGAGTT GATGAAGCAGT) and NS1R (TGGGTCAATACGAAGAACC TCATTATAG) primers were used to generate a 490-bp DNA fragment. For *sec-2*, left tDNA border (GAACATCGGTCTC AATGCA) and NS4R (AAACCAAGACAAAATCCAGATCCT CATCA) were used to generate a 720-bp fragment. For detection of the wild-type *SEC* allele corresponding to *sec-2*, GSP31 (GCTCCGATCCAGGTTTCATA) and GSP33 (ACACTTCGC CTGATATGTTCACTCTTC) primers were used to generate an 890-bp DNA fragment.

To differentiate between *SPY* and *spy-3*, a CAPs (Konieczny and Ausubel 1993) marker was used. DNA was amplified using SPY-LPD (AAAACAGTCGCCAAGCCTAACC) and JP-91 (GCG ACCTATCACCATTGGA) primers. After PCR, 5 μl of the reaction was used in standard restriction enzyme digestion with *Alu*I. The 763-bp DNA fragment of the *spy-3* allele was cut into 550- and 213-bp fragments by the restriction enzyme *Alu*I while the wild-type allele remained uncut.

RESULTS

Identification of *SEC*: The *SEC* gene was identified by searching for plant EST sequences with translational similarity to SPY and OGT proteins. This search did not identify any ESTs with identity to the carboxy-terminal catalytic domain, which is diagnostic for OGTs. The search did, however, identify several ESTs with similarity to the TPR domains, which exist in many proteins. Because the sequence information of each EST clone is generated by single-pass sequencing from the 5′ end of cloned cDNAs and the TPR domains of SPY and OGTs are >1 kb in length, the 12 Arabidopsis EST clones with the highest level of homology to SPY/OGT TPRs were ordered from the *Arabidopsis* Research Center and examined further. When grouped by cross-hybridization, three groups were identified, none of which cross-hybridized with SPY. The longest clone(s) in each of the three groups was more fully sequenced. The predicted amino acid translation of the EST H76849 sequence had similarity to the catalytic domains of both SPY and mammalian OGTs. The locus encoding EST H76849 was designated *SECRET AGENT* (*SEC*). The clone encoding W43557, which is included on the Arabidopsis Functional Genomics Consortium microarray, was found to be a chimera between *SEC* and an unrelated sequence.

The SEC EST clone was predicted to be an incomplete cDNA because the open reading frame (ORF) appeared to be incomplete and RNA-blot analysis (data not shown) determined that *SEC* mRNA was longer than the EST. Therefore, 5′ RACE was used to obtain the 5′ end of the *SEC* cDNA. A full-length *SEC* cDNA clone was constructed from the RACE cDNA and the H76849 EST.

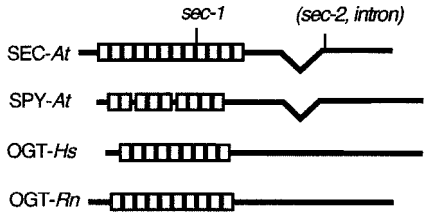

FIGURE 1.—Comparison of the protein structures of the Arabidopsis SEC and SPY proteins and the OGT proteins of human and rat. The predicted protein structures of Arabidopsis SEC (SEC-*At*, protein accession no. AAF26789.1), SPY-*At* (AAC49446; JACOBSEN *et al.* 1996), and OGTs from human (OGT-*Hs*, AAH14434; LUBAS *et al.* 1997) and rat (OGT-*Rn*, ACC53121; KREPPEL *et al.* 1997) are shown. The TPR domains are depicted as boxes, and the carboxy-terminal deletion of SEC and SPY, relative to the OGTs, is shown as a "v." The locations of insertions within the genomic sequence of *SEC* are shown relative to the translated sequence of the SEC protein. The *sec-2* insertion is within an intron.

The cDNA clone was used to identify a genomic clone and both were fully sequenced. The sequence of the genomic clone was identical with the sequence determined by the *Arabidopsis* Genome Sequence Project (GenBank accession nos. T6K12.14 and At3g04240). The 5′-RACE-derived portion of the clone was identical to the genomic sequence. The EST-derived portion of the cDNA differed from the genomic sequences at four nucleotides but the changes did not affect the amino acid sequence. At least one of these differences is present in several recently sequenced ESTs and therefore represents allelic variation occurring within the Columbia ecotype. The *SEC* gene is located on chromosome III, ~8 cM distal to *SPY* (http://www.arabidopsis.org).

The *SEC* cDNA is predicted to encode a protein of 977 amino acids with overall similarity to human and rat OGTs and SPY. The amino-terminal TPR structure of SEC is more similar to that of animal OGTs in that the TPRs are contiguous, while SPY has insertions after the second and fifth TPRs. On the other hand, there is an insertion of 109 amino acids in both SEC and SPY that is not found in animal OGTs (Figure 1).

When the carboxy-terminal domains of SEC are compared, SEC proteins are more similar to animal OGTs than to SPY. The SEC proteins share 53–59% similarity with rat and *C. elegans* OGTs (Table 1 and Figure 2), while SPY shares an equal level of similarity with SEC (35–39%) and animal OGT proteins (33–38%). The similarities between SEC and OGTs are not spread evenly throughout the carboxy-terminal domain. Regions with higher amino acid conservation (Figure 2) have been identified previously and are predicted to play a role in catalysis (ROOS and HANOVER 2000). In these regions, SEC and animal OGTs share 63% identity.

Searches of GenBank indicate that petunia, soybean, tomato, cotton, *Medicago truncatula*, maize, barley, wheat, and rice have both SEC-like and SPY-like proteins (data not shown), suggesting that they are present in all angiosperms. SEC and the SEC-like protein of maize were more similar to each other than to their corresponding SPY or SPY-like protein (Table 1), suggesting that SPY and SEC arose by gene duplication early in, or prior to, the origin of the angiosperm lineage.

SEC *O*-GlcNAc modifies itself: The OGTs that have been examined to date modify themselves. *E. coli*-expressed human OGT is GlcNAc modified and has activity toward other substrates (LUBAS and HANOVER 2000). Moreover, *E. coli* has no endogenous OGT activity. Therefore, to determine if SEC is an OGT, we prepared a Western blot containing affinity-purified *E. coli*-expressed MBP-SEC and probed it for protein GlcNAc modifications. The assay that was employed to detect GlcNAc-modified proteins transfers [^{3}H]galactose to proteins bearing modifications with terminal GlcNAc. The most heavily labeled protein in the MBP-SEC sample corresponded to the full-length fusion protein; smaller proteins, which could be breakdown products of MBP-SEC, were also labeled (Figure 3A). Considerably less labeling occurred in the lane containing MBP-TPR (a deletion derivative of MBP-SEC lacking the putative OGT catalytic domain; Figure 3A). Although the most prominently labeled protein in the MBP-TPR sample was approximately the size of MBP-TPR, it did not comigrate with it and had the

TABLE 1

Percentage amino acid similarity of the carboxy-terminal portion of SEC, SPY, and OGT proteins

	% amino acid similarity[a]				
	SPY-*Zm*	SEC-*At*	SEC-*Zm*	OGT-*Rn*	OGT-*Ce*
SPY-*At*	0.87	0.37	0.35	0.35	0.33
SPY-*Zm*		0.39	0.38	0.38	0.37
SEC-*At*			0.83	0.59	0.56
SEC-*Zm*				0.55	0.53
OGT-*Rn*					0.67

[a] The carboxy-terminal portions of proteins shown in Figure 2 were compared with the GCG OLDDISTANCES program, using the length of the shorter of the compared sequences, excluding gaps, to calculate the percentage similarity between pairs of sequences.

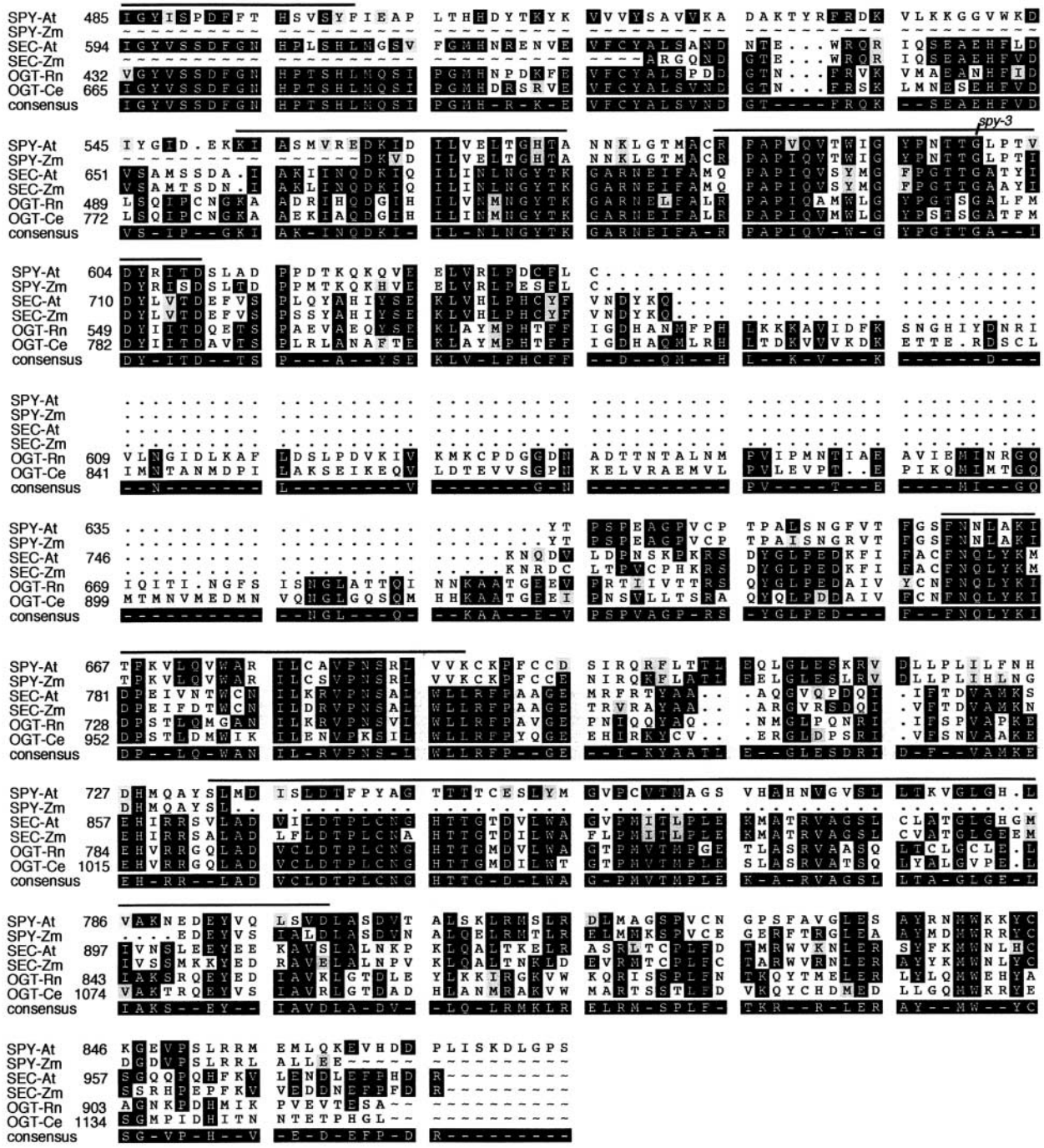

FIGURE 2.—Amino acid alignment of the carboxy-terminal domain of SEC, SPY, and OGT proteins. The SEC-*At*, SPY-*At*, OGT-*Rn*, *Caenorhabditis elegans* OGT (OGT-*Ce*, AAB63465; LUBAS *et al.* 1997), and maize SEC (SEC-*Zm*, EST accession nos. AI782882 and AW042413) and maize SPY (SPY-*Zm*; AW061770) proteins were aligned for comparison. The maize EST sequences were obtained through the *Z. maize* database (http://www.zmdb.iastate.edu) and translated by the authors using GCG software. The sequences were aligned with the GCG PILEUP program with the gap creation and extension penalties set to 20 and 2, respectively. Amino acid residues identical or similar to the consensus are shaded black and gray, respectively. Conserved regions previously identified by ROOS and HANOVER (2000) are overlined and the position of the *spy-3* mutation is indicated.

same mobility as one of the less prominently labeled proteins in the MBP-SEC sample.

To determine if the labeled modifications were *O*-linked, affinity-purified MBP-SEC and MBP-TPR preparations were labeled with [^{3}H]galactose and then subjected to β-elimination, which removes *O*-linked but not *N*-linked modifications (Figure 3B). While the majority (80%) of the labeling to the MBP-SEC preparation was *O*-linked as indicated by its release with β-elimination, the majority of the labeling in the MBP-TPR preparation was not.

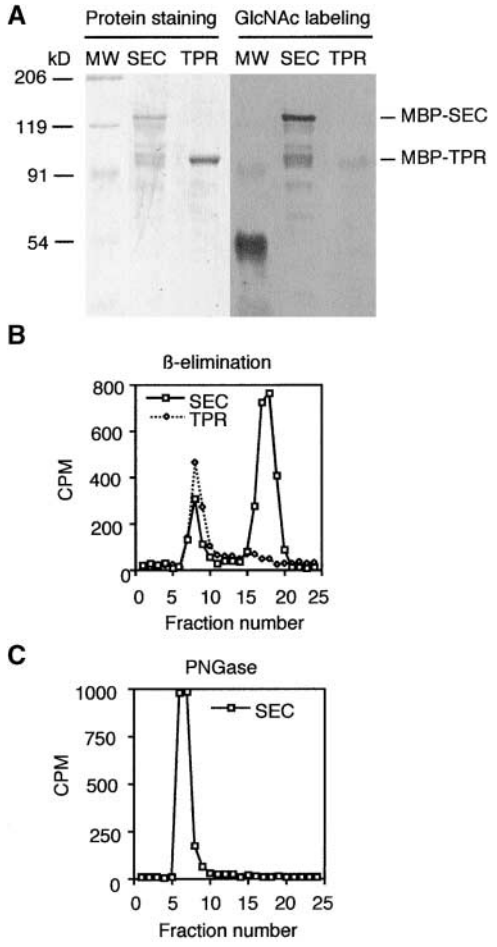

FIGURE 3.—*E. coli*-produced MBP-SEC fusion protein has an *O*-linked, terminally GlcNAcylated modification. (A) Total proteins were extracted from *E. coli* producing the MBP-SEC or MBP-TPR fusion proteins and purified with amylose-affinity chromatography. The affinity-purified proteins were resolved by SDS-PAGE on duplicate gels that were then stained with Coomassie Brilliant blue or blotted onto a nylon membrane. The membrane was blocked and allowed to react with Gal β(1-4) galactosyl-transferase (GalT) in the presence of [^{3}H]UDP-galactose, resulting in the [^{3}H]galactose labeling of terminal GlcNAc residues. The labeled GlcNAc pattern was visualized by fluorography. (B and C) Terminal GlcNAc on 100 μg each of affinity-purified MBP-SEC and MBP-TPR were treated with GalT and [^{3}H]UDP-galactose and subjected to gel filtration chromatography to remove unincorporated label. The labeled proteins were then subjected to β-elimination to remove *O*-linked modifications (B) or treated with PNGase F, which removes most *N*-linked oligosaccharide modifications (C). Following the β-elimination or PNGase treatments, the samples were subjected to gel filtration chromatography and 1-ml fractions were collected. The radioactivity within each fraction was quantitated. Proteins with terminally labeled GlcNAc eluted just after the column void volume (fraction 5). Free glycosyl groups eluted just before one column inclusion volume (fraction 24).

Furthermore the labeling of the MBP-SEC preparation was refractory to PNGase F, which hydrolyzes *N*- but not *O*-linkages (Figure 3C). These results indicate that SEC has OGT activity toward itself.

Isolation of *sec* insertional mutants: Two tDNA insertional mutants of *SEC* were identified and characterized. The site of the tDNA insertion within the *SEC* gene was determined by sequencing PCR products produced using *SEC*- and *tDNA*-specific primers. One allele, *sec-1*, has a tDNA insertion within the exon encoding the ninth TPR (Figure 1). RT-PCR analysis failed to detect *SEC* mRNA in *sec-1* plants (data not shown). A second allele, *sec-2*, contains an insertion within an intron adjacent to exons encoding the putative catalytic portion of the protein (Figure 1). Plants homozygous for either *sec-1* or *sec-2* had no obvious phenotypes. For each allele, the tDNA insertion segregated as a single Mendelian locus (data not shown), indicating that the mutations did not cause any gamete- or embryo-specific phenotypes.

Reduced transmission of linked *sec-spy* alleles: Since SEC and SPY both have OGT activity, we attempted to construct an *sec spy* double mutant that could be examined for novel phenotypes that would be consistent with these proteins having overlapping functions. Because *SEC* and *SPY* are linked on chromosome III, the scheme shown in Figure 4 was used to identify a plant in which recombination had produced a chromosome III containing *sec* and *spy*. In the first part of this scheme, plants homozygous for *spy-3* were selected by their resistance to the GA biosynthesis inhibitor paclobutrazol (PAC^R). Plants homozygous for *sec* mutant alleles were sensitive to PAC (not shown). In the second part of the scheme, Kan^R plants within the population of *spy-3* homozygotes were selected. The genotypes of these plants were determined by allele-specific PCR and CAPs markers. All of the selected plants had the genotype + *spy/sec spy*.

The + *spy/sec spy* plants were allowed to self and set seed. It was expected that 75% of the progeny seed would be Kan^R (indicating inheritance of the *sec spy* chromosome); however, only 31% of the seedlings were Kan^R (Figure 5). Similar results were obtained in crosses utilizing *sec-2* (data not shown). PCR testing of Kan^S plants indicated that the low Kan^R:Kan^S ratio was not due to incorrect phenotyping of *sec* plants carrying the Kan^R gene. The observed Kan^R:Kan^S ratio was not consistent with the simple models of either lethality of the double mutant or lethality in only one of the gametes.

Factors influencing the inheritance of *sec spy*: Because the reduced inheritance of *sec spy* could not be explained by a simple model of gamete or embryo lethality, two sets of reciprocal crosses were performed and analyzed to determine what factors were influencing *sec spy* inheritance. In the first set of reciprocal crosses, + *spy/sec spy* plants were crossed as females or males to both wild-type and *spy* plants. In the second set of crosses, + +/*sec spy* plants were crossed as females or males to both wild-type and *spy* plants. By examining the inheritance of the *sec spy* chromosome in these two sets of crosses, it was possible to estimate male and female *sec spy* gamete inheritance rates and determine whether

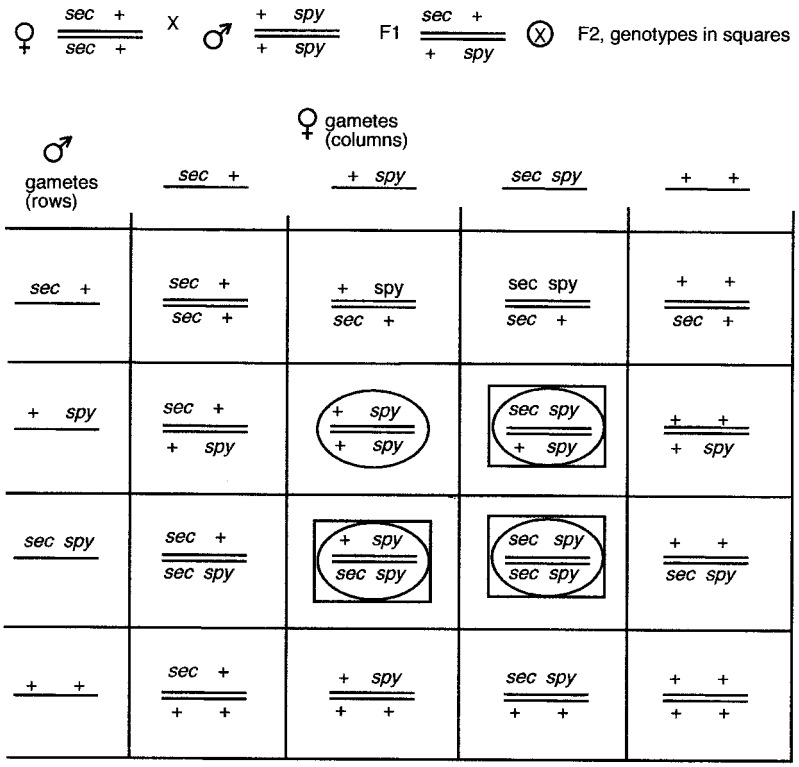

FIGURE 4.—A selection scheme was used to identify a *sec spy* chromosome. An F_2 population from a cross between *sec-1* and *spy-3* mutants was used for selecting a *sec spy* chromosome. A chromosome III is displayed for each possible F_2 gamete in the outside row and column. Recombinant and nonrecombinant chromosomes are shown. The two chromosomes III of each progeny resulting from all possible combinations of the gametes are displayed in the squares. Only plants homozygous mutant for *spy* were expected to have a PAC^R phenotype (circled genotypes). Plants that have a *sec* allele carry a tDNA insertion with a gene conferring Kan^R. Plants that had at least one *sec spy* chromosome were identified by selecting among the F_2 progeny for plants that were both PAC^R and Kan^R (boxed genotypes).

there were any parental influences on the inheritance of the *sec spy* chromosome.

When + *spy/sec spy* plants were used as male parents, only 5–7% of the resulting progeny were Kan^R (Figure 6, A and B), indicating a deficiency in the transmission of the *sec spy* chromosome through pollen.

Since we wanted to determine if the parental genotype affected the male transmission of the *sec spy* chromosome, the transmission of the *sec spy* chromosome from + +/*sec spy* plants was also examined. When + +/*sec spy* plants were used as males, there was a reduction in the number of plants that were Kan^R (31–36%; Figure 6, C and D). However, the transmission of the *sec spy* chromosome from + +/*sec spy* plants was not equal to the percentage of Kan^R progeny because recombination between *SEC* and *SPY* loci produces *sec SPY* chromosomes, which, when transmitted, also confer Kan^R. Using the observed Kan^R:Kan^S ratio from the wild type by + +/*sec spy* cross (Figure 6C) and the expected recombination rate between *SEC* and *SPY*, we estimated that the transmission rate of the s*ec spy* chromosome through the male was 26% (Figure 7). This rate of transmission (26%) was higher than that observed when + *spy/sec spy* plants were used as males (5–7%; Figure 6A and Figure 7; contingency $\chi^2 = 64.9$, $P = 7 \times 10^{-16}$), indicating that the paternal dosage of *SPY* affected the transmission of the *sec spy* chromosome.

When female *sec spy* inheritance was examined, some

Parent	Progeny	Observed Kan^R:Kan^S (%Kan^R)	Expected Kan^R:Kan^S (%Kan^R)	χ^2 (p)
+ *spy* / *sec spy* (X)	[+ *spy* / + *spy*; + *spy* / *sec spy*; *sec spy* / + *spy*; *sec spy* / *sec spy*]	65:143 (31%)	3:1(156:52) (75%)	212 ($p < 0.01$)
			2:1(138.7:69.3) (66%)	118 ($p < 0.01$)
			1:1(104:104) (50%)	29.3 ($p < 0.01$)

FIGURE 5.—The *sec spy* chromosome is inherited at a low frequency from + *spy/sec spy* plants and this inheritance cannot be explained by a simple model. The parent and progeny genotypes are described with regard to the *SEC* and *SPY* genotypes of their individual chromosomes III. A + *spy/sec spy* plant was selfed and the numbers of Kan^R and Kan^S progeny were scored. In addition, the percentages of Kan^R progeny are given in parentheses. The expected Kan^R:Kan^S ratios were calculated for models of normal inheritance (3:1), lethality of the double mutant (2:1), or lethality of one gamete (1:1). To compare the observed ratios with those predicted by the three models, the χ^2 goodness-of-fit test was used.

Cross	Parents	Progeny	Observed Kan^R:Kan^S (%Kan^R)	Expected Kan^R:Kan^S (%Kan^R)	χ^2 (p)
A.	♀ + + / + + × ♂ + *spy* / *sec spy*	+ + / + *spy*; + + / *sec spy*	31:445 (6.5%)	1:1 (238:238) (50%)	360 ($p < 0.01$)
B.	♀ + *spy* / + *spy* × ♂ + *spy* / *sec spy*	+ *spy* / + *spy*; + *spy* / *sec spy*	18:358 (4.8%)	1:1 (188:188) (50%)	307 ($p < 0.01$)
C.	♀ + + / + + × ♂ + + / *sec spy*	+ + / + +; + + / *sec spy*; + + / + *spy*; + + / *sec* +	138:305 (31%)	1:1 (221:221) (50%)	63.0 ($p < 0.01$)
D.	♀ + *spy* / + *spy* × ♂ + + / *sec spy*	+ *spy* / + +; + *spy* / *sec spy*; + *spy* / + *spy*; + *spy* / *sec* +	59:103 (36%)	1:1 (81:81) (50%)	12.0 ($p < 0.01$)

FIGURE 6.—The *sec spy* chromosome is transmitted at reduced rates through male gametes. For the indicated crosses, the inheritance of the *sec* was scored on the basis of Kan^R and compared to the inheritance expected if there is no gamete lethality. The expected and observed numbers of Kan^R and Kan^S progeny from the crosses were compared using χ^2 goodness-of-fit tests.

similarities with male inheritance were observed. There was reduced transmission of the *sec spy* chromosome when + *spy/sec spy* plants were used as females in crosses with either wild-type or + *spy*/+ *spy* male plants (Figure 8, E and F). A 50% rate of transmission was expected, but only 30% of the progeny inherited the *sec spy* chromosome. However, when + +/*sec spy* females were used, there was no deficiency in the inheritance of the *sec spy* chromosome (Figure 8, G and H; Figure 9). Therefore, the maternal gene dosage of *SPY* also affected the transmission of the *sec spy* chromosome.

Alternatively, the hypothesized parental effect on the *sec spy* transmission rate could have been due to differential lethality between + *spy/sec spy* and + +/*sec spy* embryos. However, no differential lethality was detected (Figure 6, A and B; contingency $\chi^2 = 1.2$, $P = 0.27$; Figure 6, C and D; contingency $\chi^2 = 1.5$, $P = 0.22$; Figure 8, E and F; contingency $\chi^2 = 0.03$, $P = 0.86$; Figure 8, G and H; contingency $\chi^2 = 1.8$, $P = 0.18$).

Occurrence of the *sec spy/sec spy* genotype: Since SEC or SPY was required for gamete development, we hypothesized that OGT function was also required for seed development and, as a test of this hypothesis, attempted to recover double-mutant seedlings. Although gamete lethality would reduce the recovery of double mutants, we were able to estimate that 12% of the progeny of selfed + +/*sec spy* plants would be double mutants (Figure 10A). However, when 38 progeny seedlings were genotyped by PCR, none were double mutants, indicating that the double-mutant seedlings did not occur ($P = 0.01$) at the predicted frequency. As an additional test, seeds were germinated on PAC because 70% of the PAC^R progeny from selfed + +/*sec spy* plants were expected to be double mutants (Figure 10A). However, none of the PAC^R seedlings were double mutants (Figure 10B). Furthermore, the observed genotype frequencies were consistent with double-mutant lethality. The failure to recover double mutants in any of these tests suggested a defect in the development of *sec spy/sec spy* seeds.

In tests to find a viable double mutant, we noted that 14% of the selfed seeds from + +/*sec spy* plants did not germinate, suggesting that the double mutants might be among these nongerminating seeds. In addition, an equal proportion of the desiccated seeds appeared to be misshapen. This contrasted with the low percentage (2%) of both misshapen and nongerminating seeds produced when + +/*sec spy* plants had been crossed with *spy-3* males. To determine if the misshapen seeds were double mutants with defects in embryo development, we imbibed the seeds overnight at 4°, removed seed coats, examined the embryos, and determined their genotype by PCR. Most of the misshapen seeds either did not have visible embryos (33%) or had a small clump of cells that might have been an embryo that aborted early in development (33%). We were not able to determine the genotype of these aborted embryos. However, a portion of the misshapen seeds appeared to have initiated various degrees of embryo development (13%) and were double mutants (see supplemental figure at http://www.genetics.org/supplemental). Some of these mutants were small and resembled oversized heart-shaped embryos. Others had structures that resembled roots and cotyledonary bumps or cotyledons to various degrees but none resembled wild-type-shaped embryos. If

Genotype, Estimated frequency	Phenotype	Frequency
+ + / + + (1- θ)/2	Kan^S	0.63
+ + / *sec spy* β(1- θ)/2	Kan^R	0.26
+ + / + *spy* θ/2	Kan^S	0.05
+ + / *sec* + θ/2	Kan^R	0.05

θ = recombination frequency
β = recovery rate of *sec spy* chromosome in progeny with + +/*sec spy* fathers
M = (1-θ)(1+β)/2 + θ = sum of the frequencies in column 1.

FIGURE 7.—Calculating the transmission rate of the *sec spy* chromosome from a + +/*sec spy* male parent. The first column shows each of the four possible genotypes resulting from cross C in Figure 6 and the expected frequencies for each of these genotypes is also given using the parameters defined at the bottom. The second column gives the Kan^R or Kan^S phenotypes associated with each genotype. The third column gives the calculated frequencies of each genotype. These were obtained by setting $\theta = 0.081$ [the 8% map distance between SEC and SPY predicts an 8.1% recombination rate (KOSAMBI 1944)] and by equating the observed Kan^R:Kan^S ratio from cross C in Figure 6 (138/305) to the expected value: $[\beta(1 - \theta)/2 + \theta/2]/[(1 - \theta)/2 + \theta/2]$, which can be simplified to $[\beta(1 - \theta) + \theta]$. Using these equations, β is equal to 0.405. *M* is calculated by $[(1 - \theta)/2 + \beta(1 - \theta)/2 + \theta/2 + \theta/2]$. The estimated values for θ and β were used in the equations described in the first column and each value was then divided by *M* to give the frequencies shown in the third column.

all three classes (no embryo, early, and later aborted embryos) were double mutants, then the proportion of the progeny of selfed + +/*sec spy* plants expected to be double mutants would be equal to that observed. Finally, some embryos dissected from among the misshapen seeds had a wild-type embryo shape but PCR genotyping indicated that none of these were double mutants. In all, we did not obtain any double-mutant seeds that were viable or contained embryos with a wild-type appearance.

DISCUSSION

This article describes the discovery and genetic characterization of *SEC*, a gene with predicted translational similarity to SPY and animal OGT proteins. Because the analysis of *spy* mutants suggested that plants might contain additional OGTs, we initiated a search for additional OGTs. While database searches did not identify any sequences with unambiguous similarity to OGTs, they did identify ESTs encoding TPR proteins. Since the amino-terminal halves of OGTs are composed of a series of TPR repeats, ESTs encoding TPRs with the highest similarity to OGT TPRs were sequenced further to determine if they had identity to the OGT catalytic region. This process identified a second Arabidopsis OGT that we have named *SECRET AGENT*.

Since the *SEC* EST clone was shorter than *SEC* mRNA (data not shown), 5′ RACE was performed to obtain the 5′ portion of *SEC* cDNA and a full-length cDNA clone was reconstructed from the EST and 5′-RACE product. The *SEC* cDNA clone is likely to be full length because it is the same size as *SEC* mRNA as determined by RNA blot analysis (data not shown) and because the start codon for the *SEC* ORF is the first start codon of the cDNA and is preceded by stop codons in all frames. In contrast to the situation in rats in which several OGT RNAs differ in length (KREPPEL *et al.* 1997), probing RNA blots with SEC sequences detects only a 3.3-kb RNA (data not shown).

The SEC cDNA encodes a 977-amino-acid protein with overall similarity to OGTs (Figures 1 and 2; Table 1). The carboxy-terminal amino acid sequence similarities and differences between SEC, SPY, and OGTs did not suggest a simple model for the evolutionary history of the proteins. We found that although the carboxy-terminal portions of SEC and OGTs (53–59%) were more similar to each other than to SPY, the two plant OGTs, SEC and SPY, both had a deletion of ~100 amino acids relative to animal OGTs (Figures 1 and 2; Table 1).

Among animal and plant OGTs, there is some variation in the number of TPRs, but all sequences have 9–12 TPRs (KREPPEL *et al.* 1997; LUBAS *et al.* 1997). All animal OGTs and SEC have contiguous TPR repeats; however, SPY has two insertions between adjacent TPRs. Since the TPR domains participate in protein-protein interactions (BLATCH and LASSLE 1999; TSENG *et al.* 2001), differences in the TPR domain may affect the three-dimensional α-helical structure of the TPR domain and therefore the binding of interacting proteins. The TPR motifs of animal OGTs are known to affect substrate recognition (KREPPEL and HART 1999; LUBAS and HANOVER 2000). Therefore, differences in the TPR structures of SEC and SPY may result in the proteins having different interaction partners or substrates.

The prediction, based on sequence comparisons, that SEC is a functional OGT was supported by results from a SEC protein expression experiment. MBP-SEC fusion protein isolated from *E. coli* had *O*-linked modifications bearing terminal GlcNAc (Figure 3). Since *E. coli* does not have endogenous OGT activity (LUBAS and HANOVER 2000), SEC must be *O*-GlcNAc modifying itself. This is not surprising; when human OGT protein was expressed in *E. coli* it was also found to modify itself (LUBAS and HANOVER 2000). Experiments are in progress to determine if, like animal OGTs, each modification on SEC consists of a single GlcNAc. In addition,

Cross	Parents	Progeny	Observed Kan^R:Kan^S (%Kan^R)	Expected Kan^R:Kan^S (%Kan^R)	χ^2 (p)
E.	♀ + spy / sec spy × ♂ + + / + +	+ spy / + + ; sec spy / + +	61:143 (30%)	1:1 (102:102) (50%)	32.9 ($p < 0.01$)
F.	♀ + spy / sec spy × ♂ + spy / + spy	+ spy / + spy ; sec spy / + spy	72:163 (31%)	1:1 (117.5:117.5) (50%)	35.2 ($p < 0.01$)
G.	♀ + + / sec spy × ♂ + + / + +	+ + / + + ; sec spy / + + ; + spy / + + ; sec + / + +	142:147 (49%)	1:1 (144.5:144.5) (50%)	0.1 (0.75)
H.	♀ + + / sec spy × ♂ + spy / + spy	+ + / + spy ; sec spy / + spy ; + spy / + spy ; sec + / + spy	129:167 (44%)	1:1 (148:148) (50%)	4.9 (0.02)

FIGURE 8.—The *sec spy* chromosome is transmitted at reduced rates through female gametes. For the indicated crosses, the inheritance of the *sec* was scored on the basis of Kan^R and compared to the inheritance expected if there is no gamete lethality. The expected and observed numbers of Kan^R and Kan^S progeny from the crosses were compared using χ^2 goodness-of-fit tests.

experiments are in progress to determine if it has activity toward other proteins.

To determine the function of *SEC* in plants, two tDNA insertional mutants of *sec* were identified, but these mutants did not have obvious phenotypes. Only when a chromosome III containing mutations in both *sec* and *spy* was identified did we detect phenotypes. When a + *spy*/*sec spy* plant was selfed, there was a deficiency in the frequency of progeny inheriting the *sec spy* chromosome (Figure 5). The mechanism for this deficiency was investigated in a series of reciprocal crossing experiments that indicated that the transmission of the *sec spy* chromosome through both male and female gametes was reduced (Figures 6–9). Furthermore, the dosage of *SPY* in the parent strongly affected the penetrance of gamete lethality. We hypothesize that the SEC or SPY proteins or *O*-GlcNAcylated products needed for gamete development can be supplied by parental tissues and that parents with a higher dosage of *SEC* and *SPY* provide more of the limiting factor(s), thereby reducing the penetrance of the synthetic lethal phenotype.

Genotype, Estimated frequency	Phenotype	Frequency
+ + / + + (1- θ)/2	Kan^S	0.47
sec spy / + + α (1- θ)/2	Kan^R	0.45
+ spy / + + θ/2	Kan^S	0.04
sec + / + + θ/2	Kan^R	0.04

θ = recombination frequency
α = recovery rate of *sec spy* chromosome in progeny with + +/*sec spy* fathers
$F = (1-\theta)(1+\beta)/2 + \theta$ = sum of the frequencies in column 1.

FIGURE 9.—Calculating the transmission rate of the *sec spy* chromosome from a + +/*sec spy* female parent. The third column shows the frequency for each of the possible genotypes resulting from the cross shown in Figure 8G. See Figure 7 for a description of the equations and calculations used. The calculated values for α and *F* were 0.96 and 0.73, respectively.

Parental suppression of lethal gametophytic mutations may be a common phenomenon in plants. BONHOMME *et al.* (1998) observed a low recovery of gametophytic mutations from tDNA insertional lines. It was expected that such mutations would be frequent given the prediction that 60–80% of all plant genes are expressed in the male gametophyte (WILLING *et al.* 1988; MASCARENHAS 1990). BONHOMME *et al.* (1998) postulated that both gene redundancy and "metabolic supplementation" could account for the paucity of gametophytic lethals. One recent example of maternal supplementation was observed in studies with the *PROLIFERA* (*PRL*) gene of Arabidopsis (SPRINGER *et al.* 2000). The *PRL* gene encodes an MCM-like protein, known in yeast and animal systems to be involved in DNA replication. Loss of *prl* was embryo lethal, but not completely lethal for

A

+ + / sec spy ⊗ Progeny genotypes and frequency of genotypes in squares

♀ gametes (columns)

♂ gametes (rows)

	+ + (1- θ)/2F	sec spy α(1- θ)/2F	+ spy θ/2F	sec + θ/2F
+ + (1- θ)/2M	+ + / + + 0.30	sec spy / + + 0.30	+ spy / + + 0.02	sec + / + + 0.02
sec spy β(1- θ)/2M	+ + / sec spy 0.12	(sec spy / sec spy) 0.12	(+ spy / sec spy) 0.03	sec + / sec spy 0.03
+ spy θ/2M	+ + / + spy 0.02	(sec spy / + spy) 0.02	(+ spy / + spy) 0.002	sec + / + spy 0.002
sec + θ/2M	+ + / sec + 0.02	sec spy / sec + 0.02	+ spy / sec + 0.002	sec + / sec + 0.002

B

	Total	PAC^R: sec spy / sec spy	PAC^R: [+ spy / sec spy ; sec spy / + spy]	PAC^R: + spy / + spy	χ^2 (p)
Observed	355	0	20	2	
Expected, double mutant viable		42.6	17.8	0.7	45.3 ($p < 0.01$)
Expected, double mutant lethal		0	20.2	0.8	1.8 (0.18)

FIGURE 10.—The *sec spy/sec spy* genotype was not present among the PAC^R progeny of selfed + +/*sec spy* plants. (A) Using the estimates of α, β, *F*, and *M* determined in Figures 7 and 9 and the recombination rate (θ = 0.081), the frequency of each progeny genotype produced by selfing a + +/*sec spy* plant was calculated. Genotypes that were expected to be PAC^R are circled. (B) The PAC^R plants from among the progeny of a selfed + +/*sec spy* plant were genotyped by PCR. The total number of seeds screened and the number of plants identified for each genotypic class are listed in the "observed" row. The expected number of progeny for each genotypic class was calculated for both double-mutant viability and lethality. The observed number of plants in each genotype was compared to the expected number using χ^2 goodness-of-fit tests.

the female gametophyte. Maternal supplementation was suggested as one possible explanation for the incompletely penetrant gametophytic lethality of *prl*. An alternative hypothesis is that maternal or paternal effects are caused by imprinting of the respective alleles; however, imprinting gives a differential effect on either maternal or paternal gametes (KINOSHITA *et al.* 1999; LUO *et al.* 2000). Because both gametes are affected by loss of SEC and SPY, imprinting is not a likely explanation for the parental affects observed in these experiments.

The carryover of parental SEC, SPY, or *O*-GlcNAcylated substrates may have also contributed to the phenotypes observed for double-mutant embryos. Double-mutant embryos aborted at various stages of development with none completing embryogenesis and producing viable seeds, suggesting that parental supplementation can partially support embryo development.

The synthetic interaction between *sec* and *spy* suggests that OGT activity and protein *O*-GlcNAcylation are essential for gamete and seed development. Deletion of the mouse *OGT* gene is lethal (SHAFI *et al.* 2000). Therefore, protein *O*-GlcNAcylation is likely to be an essential modification in both plants and animals. Interestingly and in contrast to animals, which have one *OGT*, searches of GenBank have identified both *SEC*- and *SPY*-like genes in petunia, soybean, tomato, cotton, *M. truncatula*, maize, barley, and wheat, suggesting that all angiosperms have two OGTs. These searches have not identified any other candidate OGTs, suggesting that plants have only two OGTs.

The presence of two OGTs in plants raises the possibility that each has a specialized function(s). This hypothesis is supported by the observation that *spy* plants exhibit phenotypes while *sec* plants have no obvious phenotypes. While multiple *spy* alleles have been recovered in independent screens for suppressors of GA deficiency or

reduced GA response (Jacobsen and Olszewski 1993; Wilson and Somerville 1995; Silverstone *et al.* 1997), *sec* mutants were not recovered in these screens. These observations do not rule out the possibility that *sec* plays a minor role in GA signaling. Therefore, characterizations of *sec* mutants to determine if they have subtle defects in GA signaling are ongoing. This work also suggests that SPY has additional functions in plant development that have not been revealed in previous studies involving the analysis of *spy* plants. Future experiments are aimed at determining the possible unique functions of SEC as well as the essential functions carried out by both SEC and SPY proteins.

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