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Novel Nucleocytoplasmic Protein *O*-Fucosylation by SPINDLY Regulates Diverse Developmental Processes in Plants

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Abstract

In metazoans, protein *O*-fucosylation of Ser/Thr residues was only found in secreted or cell surface proteins, and this post-translational modification is catalyzed by ER-localized protein O-fucosyltransferases (POFUTs) in the GT65 family. Recently, a novel nucleocytoplasmic POFUT, SPINDLY (SPY), was identified in the reference plant *Arabidopsis thaliana* to modify nuclear transcription regulators DELLAs, revealing a new regulatory mechanism for gene expression. The paralog of AtSPY, SECRET AGENT (SEC), is an *O*-link-*N*-acetylglucosamine (GlcNAc) transferase (OGT), which *O*-GlcNAcylates Ser/Thr residues of target proteins. Both AtSPY and AtSEC are tetratricopeptide repeat-domain-containing glycosyltransferases in the GT41 family. The discovery that AtSPY is a POFUT clarified decades of miss-classification of AtSPY as an OGT. SPY and SEC play pleiotropic roles in plant development, and the interactions between SPY and SEC are complex. *SPY-like* genes are conserved in diverse organisms, except in fungi and metazoans, suggesting that *O*-fucosylation is a common mechanism in modulating intracellular protein functions.

Graphical Abstract

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Authorship

All listed authors meet the ICMJE criteria. We attest that all authors contributed significantly to the creation of this manuscript, each having fulfilled criteria as established by the ICMJE.

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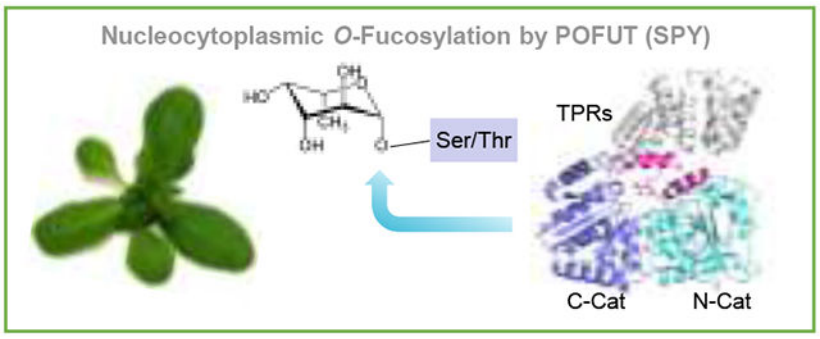
IRB approval was obtained (required for studies and series of 3 or more cases)

Written consent to publish potentially identifying information, such as details or the case and photographs, was obtained from the patient(s) or their legal guardian(s).

Conflict of Interest

No conflict of interest exists



Keywords

protein *O*-fucosylation; protein *O*-GlcNAcylation; nucleocytoplasmic protein *O*-fucosylation; SPINDLY; POFUT

Discovery of nucleocytoplasmic protein *O*-fucosyltransferase SPINDLY in plants

The discovery of the nucleocytoplasmic protein *O*-fucosyltransferase (POFUT) came from the studies of SPINDLY (SPY) in Arabidopsis. AtSPY was initially identified as a negative regulator of plant hormone gibberellin (GA) signaling because the hypomorphic mutations in *AtSPY* partially rescue the seed germination defect and dwarf phenotypes caused by chemical-induced GA deficiency or genetic mutations in GA biosynthesis [1,2]. In addition, SPY represses other aspects of GA-regulated processes, including floral induction, anther development and pollen tube growth [1-3].

Based on sequence comparison, both AtSPY and its paralog AtSEC (SECRET AGENT) were predicted to be OGTs, with a tetratricopeptide-repeat (TPR) domain and a putative OGT catalytic domain (Fig. 1A) [4-7]. The TPR domain of SPY and SEC functions as a protein-protein interaction domain for recruiting target proteins, and overexpression of the TPR domain of AtSPY has a dominant negative effect that confers a *spy*-like phenotype [8,9]. Recombinant AtSEC expressed in *E. coli* was shown to exhibit OGT activity [5], but the enzymatic activity of AtSPY was not detected conclusively in a similar in vitro assay.

Because *spy* displays elevated GA signaling, and the presence of putative *O*-GlcNAc sites in the nuclear DELLA repressors (also known as GA-signaling repressors), AtSPY was long proposed to activate AtDELLAs by *O*-GlcNAcylation [10-12]. Through a combination of electron transfer dissociation (ETD)-MS/MS analysis, in vitro enzyme assays and genetic studies, AtSEC was shown to be an OGT that *O*-GlcNAcylates DELLAs using UDP-GlcNAc as its donor substrate [13]. Surprisingly, AtSPY was found to be a novel POFUT, which is highly selective to GDP-fucose as its donor substrate and catalyzes the transfer of *O*-Fucose monosaccharide to the hydroxyl oxygen on Ser and Thr residues of DELLA proteins (acceptor substrates) [14] (Fig. 2).

Predicted 3D structure of AtSPY is unrelated to ER-localized POFUTs, but is similar to OGTs

Sequence alignment and three dimension (3D) protein structure modeling indicate that AtSPY is distinct from the ER-localized POFUTs, which belong to GlycosylTransferase Family 65 (GT65 [15], http://www.cazy.org) and modify secreted cell surface proteins in animals [16-18], Instead, AtSPY's 3D model is highly similar to the TPR domain-containing OGTs, members of the GT41 family [14,19] (Fig. 1B). Moreover, like OGTs, AtSPY is localized to both cytoplasm and nucleus [20]. AtSPY is the first nucleocytoplasmic-localized POFUT found in any organism.

Multiple *O*-Fuc and *O*-GlcNAc sites identified in AtDELLA are clustered within two structurally disordered polyS/T sequences flanking the conserved DELLA domain [13,14], suggesting that AtSPY and AtSEC may modify target proteins via a similar mechanism as in HsOGT, which modifies flexible sequences of its target proteins by binding to the substrate amide backbone [19,21]. The critical residue(s) that contribute to the distinct substrate selectivity of AtSPY have not been identified experimentally, although some differences between AtSPY and OGTs have been noted through sequence alignment and 3D model comparison. The H3 transition helix and the H1 and H2 helices of N-Cat are more divergent in AtSPY (Fig 1B). Moreover, two key His residues (H498-H499 in HsOGT and F540-H541 in AtSEC) that are crucial for OGT activity, are absent in AtSPY [13,14,19,22,23].

Opposing roles of AtSPY and AtSEC in regulating DELLA function and activities of multiple signaling pathways

Intriguingly, genetic and biochemical studies further showed that *O*-GlcNAc and *O*-Fuc modifications by the two paralogs AtSEC and AtSPY display opposite effects on DELLA function and GA signaling activity [13,14]. DELLAs are master growth repressors, which integrate multiple signaling activities by protein-protein interactions with key transcription factors to coordinate plant growth with internal and external cues [12,24]. For example, BRASSINAZOLE-RESISTANT1 (BZR1) and PHYTOCHROME-INTERACTING-FACTORs (e.g., PIF3 and PIF4) are key transcription factors that promote hypocotyl elongation in response to the phytohormone brassinosteroid (BR) and external light conditions, whereas DELLAs inhibit hypocotyl growth by antagonistic interactions with BZR1 and PIFs to repress expression of BZR1- and PIFs-target genes [25-27]. The null *sec* mutant shows reduced GA responses with a shorter hypocotyl and internode length than the wild-type Arabidopsis plant [13]. These results indicate that AtSEC is an activator of GA signaling, which is in contrast to the repressive role of AtSPY in GA signaling. By deduction, AtSEC may reduce DELLA activity and AtSPY may increase DELLA activity to achieve their effects on GA signaling activity. Indeed, in vitro pulldown assays showed that *O*-fucosylation by AtSPY enhances DELLA interactions with BZR1 and PIFs [14]. In contrast, *O*-GlcNAcylation by AtSEC reduces DELLA interactions with these key transcription factors in BR and light signaling pathways [13]. Furthermore, *spy* mutations confer increased responses to BR and elevated transcript levels of target genes of BZR1 and PIFs, whereas the *sec* null allele shows an opposite effect. Therefore, these two distinct *O*-

glycosyl modifications of DELLAs by AtSPY and AtSEC differentially modulate GA, BR and light signaling pathways to regulate plant growth and development. The identified *O*-GlcNAc and *O*-Fuc sites in DELLA are partially overlapping or nearby. A model was proposed in which highly *O*-GlcNAcylated DELLA may lock into a “closed form” that interferes with binding of target proteins. Increasing *O*-fucosylation may convert DELLA conformation to an “open form” that enhances interaction with target proteins (Fig. 2C).

It is unclear how SPY and SEC activities are regulated, although they appear to be unaffected by the GA status in the plant [13,14]. In animals, OGT functions as a nutrient sensor because its activity is tightly correlated with the levels of its donor substrate UDP-GlcNAc, which is derived from several key metabolites in the cell via the hexosamine biosynthesis pathway [7,28,29]. It was proposed that dynamic *O*-GlcNAc vs *O*-Fuc modifications of DELLAs (and additional regulatory proteins) may help to coordinate the metabolic status of the plant with its growth and development in response to internal and external cues, although specific glucosidases have not been identified [14].

Protein *O*-fucosylation and *O*-GlcNAcylation play diverse roles in plant development

The interplay between AtSPY and AtSEC during Arabidopsis development is complex. Although SPY and SEC play opposite roles in regulating DELLA-mediated signaling activities as described above, these two protein glycosyltransferases may interact differently in DELLA-independent cellular processes in plants. Both AtSPY and AtSEC regulate embryogenesis and flowering time [1,5,30], whereas each enzyme displays unique roles in a subset of developmental processes. For example, SPY is a positive regulator of phytohormone cytokinin signaling [31,32], and regulates the circadian clock [33,34]. In contrast, the *sec* mutations do not alter cytokinin responses or circadian rhythms. On the other hand, SEC but not SPY promotes Plum Pox Virus (PPV) infection by *O*-GlcNAcylating the coat protein of PPV [35,36]. In vitro assays suggest that *O*-GlcNAcylation regulates protein trafficking through plasmodesmata by altering their interactions with the *Nicotiana tabacum* NON-CELL-AUTONOMOUS PATHWAY PROTEIN1 [37]. Mechanisms of cellular processes regulated by both SPY and SEC, and those that are uniquely regulated by SPY are described below.

Embryo development

While AtSPY and AtSEC play opposite roles in regulating DELLA to modulate multiple signaling activities [13,14], the *spy sec* double mutant is embryo lethal [5,38]. This synthetic lethal phenotype of *spy sec* indicates that AtSPY and AtSEC regulate unidentified essential process(es) during embryogenesis. The knockout OGT mutants in mouse and in Drosophila are embryo lethal [39-41]. The OGTs in animals are known to regulate intracellular functions including altering gene expression at the epigenetic and transcription levels as well as modulating protein synthesis, stability, activity or subcellular localization [42,43]. In contrast, the functions of OGT (SEC) and POFUT (SPY) in plants are much less understood. Recently, proteomic studies have identified a large number of *O*-GlcNAcylated proteins in Arabidopsis (262) and in winter wheat *Triticum aestivum* (168), many of which function in

epigenetic and transcriptional regulation, RNA processing, translation and metabolic processes [44,45], suggesting that OGT in plants also play diverse roles as the animal OGTs do. So far, the known protein substrates of AtSPY only include DELLAs and PSEUDO RESPONSE REGULATOR 5 (PRR5, a circadian clock component) (see below), although several other interacting proteins have been identified by Y2H or co-IP assays (including MYB, NAC-like, TCP and ZIM domain transcription factors, a circadian clock regulator GIGANTIA, and SWI3C, a subunit of the chromatin remodeling complexes) [33,46-49]. Considering that *O*-Fuc and *O*-GlcNAc sites in DELLA largely overlap [13,14], SPY and SEC may share additional common targets in plants. However, the interaction between *O*-GlcNAcylation and *O*-fucosylation may be different depending on the target proteins because the embryo-lethal phenotype of the *spy sec* mutant suggests an additive interaction, which is in contrast to their antagonistic interaction in modulating DELLA activity. In addition, AtSPY plays unique roles in a subset of cellular processes, which will be discussed below.

Flowering time

The hypomorphic *spy* mutants in Arabidopsis flower earlier than WT in both long-day and short-day conditions, indicating that AtSPY negatively regulates floral induction [1,10]. One way for AtSPY to delay flowering is by enhancing DELLA activity to repress GA-induced flowering. Additionally, AtSPY interacts with a core circadian clock protein GIGANTIA (GI) that promotes flowering in long day [33]. The *gi* mutant is late flowering, whereas *spy gi* double mutant is early flowering, indicating that *spy* is epistatic to *gi*, although the role of SPY-GI interaction in flowering time control is unclear. Interestingly, *O*-GlcNAcylation catalyzed by AtSEC also delays flowering in Arabidopsis. AtSEC upregulates the expression of the major flowering repressor *FLOWERING LOCUS C* (*FLC*) [30]. Further analysis of the chromatin around the *FLC* locus indicates that H3 lysine 4 trimethylation (H3K4me3, an active chromatin mark) is reduced significantly in the *sec* mutant. Importantly, AtSEC *O*-GlcNAcylates the histone methyltransferase ATX1 in planta, and this modification enhances ATX1's activity to methylate H3 in vitro. Thus, AtSEC induces expression of *FLC* to delay flowering, at least in part by *O*-GlcNAcylation and activation of the histone methyltransferase ATX1 to increase the H3K4me3 active chromatin mark at the *FLC* locus. In contrast, *O*-GlcNAcylation in *Triticum aestivum* (winter wheat) mediates vernalization-induced flowering [50]. Vernalization (prolonged cold period) promotes flowering in winter wheat by enhancing expression of a flowering activator TaVRN1 (a MADS-box transcription factor). Without vernalization, TaVRN1 mRNA processing is inhibited by an RNA binding protein TaGRP2 that binds to the first intron of TaVRN1 pre-mRNA. Vernalization increases TaVRN1 mRNA levels by inducing *O*-GlcNAcylation of TaGRP2, which in turn promotes sequestration of TaGRP2 by a vernalization-induced lectin VERN2.

Cytokinin responses

In addition to an elevated GA-response phenotype, the *spy* single mutants in Arabidopsis display other pleiotropic phenotypes, including abnormal cotyledon numbers, altered phyllotaxy, reduced leaf serration, and decreased trichomes on sepals [10,32,51]. The reduced leaf serration and sepal trichome formation in *spy* mutants are caused by reduced responses to another phytohormone cytokinin, indicating that AtSPY is a positive regulator

of cytokinin signaling [31,32]. Screening and characterization of AtSPY-interacting proteins identified two bHLH transcription factors TCP14 and TCP15 that are involved in AtSPY-regulated cytokinin responses [49]. The *tcp14 tcp15* double mutant shows reduced cytokinin responses, whereas overexpression of TCP14 displays enhanced cytokinin responses. The GFP-TCP14 protein accumulates to a lower level in the *spy* mutant than that in WT, but this reduced protein stability of TCP14 can be reversed by treatment with the 26S proteasome inhibitor MG132 or in the mutant *cul1* background (*CULLIN1* encodes a component of the SCF E3 ligase complex) [52]. It is likely that AtSPY stabilizes TCP14 by *O*-fucosylation, although this has not been demonstrated directly.

Circadian clock

The animal OGTs have been shown to regulate the circadian clock by rhythmic *O*-GlcNAcylation of key components of the clock. In Drosophila and mammals, the transcription repressor PERIOD binds to and inhibits transcription activators CLOCK and BMAL, whereas CLOCK/BMAL induces transcription of PERIOD. *O*-GlcNAcylation of PERIOD by OGT inhibits the activity of PERIOD by promoting its degradation and preventing its translocation to the nucleus [53,54]. In addition, *O*-GlcNAcylation of BMAL and CLOCK stabilizes these transcription activators [55]. Reducing OGT expression results in a longer circadian period [53]. OGT in Arabidopsis, however, does not play a significant role in regulating the circadian clock as the *sec* mutants do not show abnormal circadian phenotypes [34]. Instead, AtSPY was found to regulate circadian clock. The *spy* mutants display a longer circadian period in comparison to that of WT [33,34]. The circadian period phenotype of *spy* is rescued more effectively by *PSPY:GFP-SPY-NLS* (nuclear localization signal) than by *PSPY:GFP-SPY-NES* (nuclear export signal), suggesting that AtSPY mainly functions in the nucleus to modulate circadian clock speed. Intriguingly, expression of the cytoplasmic SPY fusion protein (GFP-SPY-NES) is required to inhibit GA responses (e.g., in seed germination, leaf expansion, floral induction) and to promote cytokinin signaling (leaf serration) [31,34]. These results suggest that cytoplasmic-and nuclear-localized AtSPY regulates distinct cellular responses, although it remains to be determined whether DELLAs and TCP14 are *O*-fucosylated in the cytoplasm or nucleus.

How does AtSPY regulate circadian clock? PRR5, a transcription repressor that is a key circadian clock component, was identified recently to be an interactor of AtSPY by MS analysis of proteins that were co-immunoprecipitated with SPY using *PSPY:GFP-SPY* and *PSPY:GFP-SPY-NLS* transgenic lines [34]. The*prr5* mutant shows a reduced circadian period, and*prr5* partially rescues the longer circadian period phenotype of *spy*, whereas overexpression of *PRR5* confers a longer circadian period. Transient co-expression of PRR5 and AtSPY in *N. benthamiana* showed that PRR5 is *O*-fucosylated by AtSPY. In addition, PRR5 protein levels are elevated in the *spy* mutant. Taken together, these results indicate that the nuclear-localized AtSPY modulates circadian clock speed by promoting PRR5 degradation via *O*-fucosylation (Fig. 3).

Plant architecture

Alteration of SPY function in Arabidopsis and petunia has been shown to affect plant height and leaf shape through changes in GA and cytokinin signaling activities [9,10,32]. In

addition, RNAi silencing of *SPINDLY* in *Oryza sativa* (*OsSPY* in rice) results in an increase in the leaf angle (due to increased bending of the lamina joint), which resembles an elevated BR response [56]. The *OsSPY* knockdown plants accumulate slightly elevated BR levels, suggesting that OsSPY may inhibit BR biosynthesis. If OsSPY and AtSPY are functionally conserved, it is also possible that OsSPY may repress BR signaling by enhancing DELLA-BZR1 interaction in rice.

A recent genome-wide association study (GWAS) identified OsSPY as a key factor in regulating rice architecture, including stem (culm) height, and size and numbers of panicles (branched flower clusters) [57]. In comparison to haplotype I, two polymorphisms in haplotype II, which result in S9T and R833L substitutions in OsSPY, correlate with taller stem and increased panicle size, but lower numbers of panicles. Importantly, the R833L substitution in the conserved POFUT catalytic domain was shown to reduce OsSPY activity by an in vitro enzyme assay. Further studies revealed that the effect of altered OsSPY activity on rice architecture is mainly through its regulation of GA signaling. This GWAS analysis also indicates that the enhanced *OsSPY* allele with R833 (in haplotype I) has a selective advantage through recent breeding programs because it confers a semidwarf and larger panicle-number phenotype in response to chemical fertilizer.

Root development

The *spy* mutants show root development defects, including formation of premature middle cortex (an extra layer of cortex) [58], and ectopic root hairs [59]. SPY may inhibit extra cortex formation by modulating redox homeostasis in the root meristem and elongation zone because H_2O_2 induces middle cortex formation in WT seedlings and the *spy* mutants accumulate higher amounts of H_2O_2 in their root tips than WT [60]. The precise mechanism of SPY-regulated root hair cell patterning is unclear, although SPY functions upstream of WEREWOLF and GLABRA2, which are two transcription factors that promote non-hair cell fate in the developing epidermal cells of the root [59].

Abiotic and biotic stresses

In addition to regulation of plant development, SPY also functions in plant's responses to abiotic and biotic stresses. The hypomorphic *spy* mutants in Arabidopsis are more tolerant to high salt and drought conditions than WT, whereas *SPY* overexpression confers reduced drought tolerance [61]. These results suggest that SPY negatively regulates plant's responses to these abiotic stresses. On the other hand, the *spy* mutants display enhanced susceptibility to a bacterial pathogen, *P. syringae* [62]. The quadruple *della* mutant, however, was previously shown to be more resistant to this pathogen infection [63], suggesting that SPY promotes plant defense responses by regulating pathways that are independent of GA and DELLAs.

SPY orthologs are present in diverse organisms

Phylogenetic analysis indicates that *SPY-like* genes are evolutionarily conserved, and are found in diverse organisms, including prokaryotes, protists, algae and all plants [4]. Both *SPY* and *SEC* genes are present in genomes of all plants, and in red algae. Different lineages

of bacteria and protists contain either a *SPY-like* or a *SEC-like* gene [4]. Intriguingly, animal and fungi kingdoms only contain *SEC-like* (OGT) genes, but not the *SPY-like* genes. In addition, protein *O*-GlcNAcylation by OGT in animals is a dynamic modification that is reversible by *O*-GlcNAcase (OGA), whereas no OGA orthologs have been identified in plant genomes.

Although SPY orthologs have long been assumed to be OGTs based on sequence similarity, the finding that AtSPY is a POFUT raised the question whether SPY-like proteins in non-plant organisms are also POFUTs. Consistent with this hypothesis, recent studies in *Toxoplasma gondii* (a parasitic protist) indicate that *TgSPY* also encodes a POFUT [64]. Phylogenetic analysis suggests that *TgSPY* is a *SPY-like* gene [4]. Importantly, Bendini et al. (2016) identified *O*-fucosylated nucleocytoplasmic proteins in *T. gondii* by affinity purification using a terminal fucose-specific *Aleuria aurantia* lectin (AAL) and MS/MS analysis [65]. Predicted functions of these *O*-fucosylated proteins in *T. gondii* include nucleoporins, transcription regulators, and components involved in mRNA processing and signaling, suggesting that *O*-fucosylation may regulate nuclear targeting and gene expression in *T. gondii* [65]. The knockout *TgSPY* mutant generated by CRISPR-Cas9 approach failed to exhibit any intracellular signals by AAL staining [66]. Furthermore, the POFUT activity of TgSPY was demonstrated recently by in vitro enzyme assays [67]. Mutant analysis further showed that TgSPY plays a role in promoting the accumulation of its target proteins, and *T. gondii* proliferation in vitro and in mice.

Besides AtSPY and TgSPY, two additional SPY-like proteins from *Cryptosporidium parvum* (a parasitic protist) and *Synechococcus elongatus* (a cyanobacterium) have been reported in earlier studies to hydrolyze UDP-GlcNAc in vitro, although the specific glycosyltransferase activity was not demonstrated directly [68,69]. Another study reported the crystal structures of TtOGT in *Thermobaculum terrenum* (a thermophilic bacterium) and the TtOGT-UDP complex [70]. However, TtOGT did not exhibit any OGT activity in vitro, and MS analysis of the *T. terrenum* proteome failed to identify any O-GlcNAcylated proteins. Sequence alignment suggests that TtOGT is more similar to SPYs than to OGTs [14]. It remains to be determined whether these SPY-like proteins are POFUTs. Alternatively, they may display both OGT and POFUT activities, or novel glycosyltransferase activity with distinct donor substrate selectivity.

Future perspectives

The discovery of AtSPY-catalyzed protein *O*-fucosylation reveals a novel mechanism for regulating nucleocytoplasmic protein functions in plants. Our understanding of SPY- and SEC-regulated plant growth and development is only the tip of the iceberg. Future studies using multifaceted approaches including proteomics, chemical biology, genomics and metabolomics will help to elucidate the global functions of SPY and SEC, and the interplay between protein *O*-fucosylation and *O*-GlcNAcylation in regulating plant development. In addition, it is important to determine whether and how *O*-GlcNAc and *O*-Fuc modifications serve as sensors of metabolic status in plants and how these PTMs are modulated to integrate external conditions with internal programs. Recent characterization of TgSPY, the AtSPY ortholog in the human parasite *T. gondii*, supports the notion that intracellular protein *O*-

fucosylation by SPY orthologs may regulate a wide range of biological processes in diverse organisms. The knowledge gain from studying how SPY functions in plants has broader implication in illuminating the molecular mechanisms by which nucleocytoplasmic protein *O*-fucosylation regulates gene expression and other cellular processes.

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• of special interest

•• of outstanding interest

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AtSEC (an OGT), AtSPY was shown in this study to be a novel nucleocytoplasmic POFUT. AtSPY and AtSEC play opposite roles in modulating multiple signaling pathways by affecting the activity of nuclear growth repressors DELLAs, revealing a new regulatory mechanism of gene expression.

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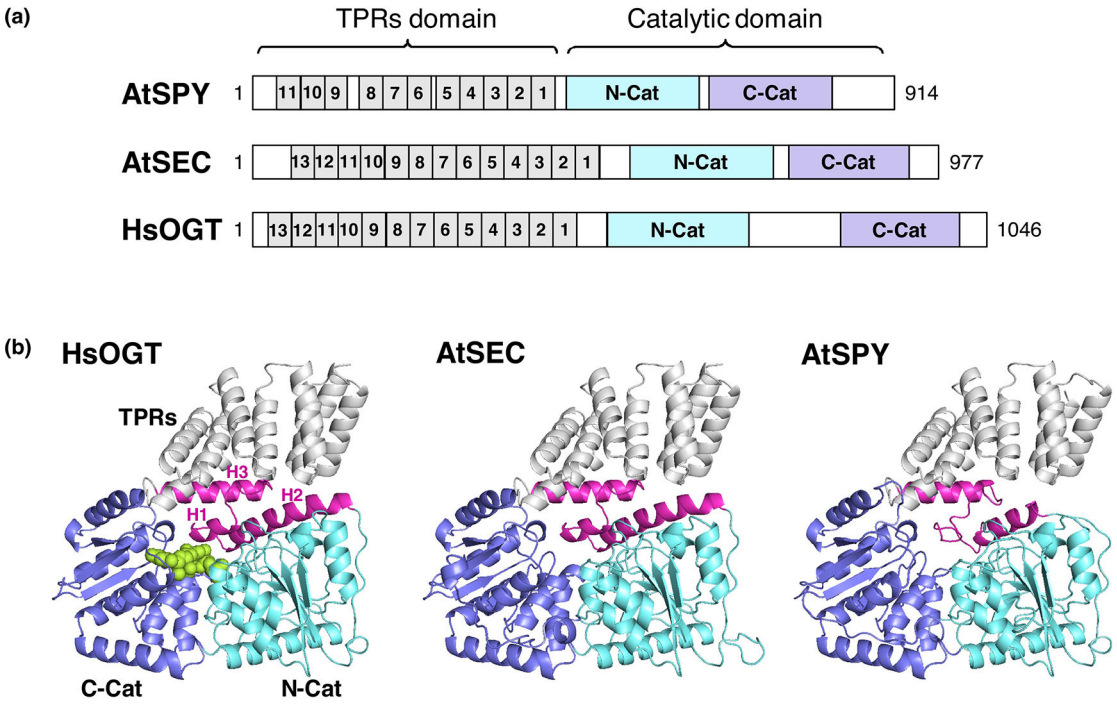

Figure 1. Structure comparison among human OGT, Arabidopsis SEC and SPY.

(**a**) Diagrams of HsOGT, AtSEC and AtSPY. TPRs are in grey. N-terminal catalytic domains, N-Cat, are in cyan. C-terminal catalytic domains, C-Cat, are in blue. (**b**) 3D structures of HsOGT (PDB ID: 4N3C, containing 4.5-TPRs)[71], and predicted 3D structures of Arabidopsis SEC and SPY using SWISS MODEL[72,73]. The HsOGT crystal structure (PDB ID: 4N3C)[71] was used as scaffold to predict AtSEC and AtSPY structures. The color schemes for HsOGT, AtSEC and AtSPY are as in (a). In (b), UDP-GlcNAc in HsOGT is shown as spheres (in lime-green). In the HsOGT structure in (b), the transitional helix (H3) between TPRs and N-Cat, and the first 2 α-helices (H1 and H2) of N-Cat are highlighted in magenta. The long intervening domain between N-Cat and C-Cat of HsOGT is omitted from the structure because this domain is uniquely present in the animal OGTs. This figure was modified from Zentella et al. [14].

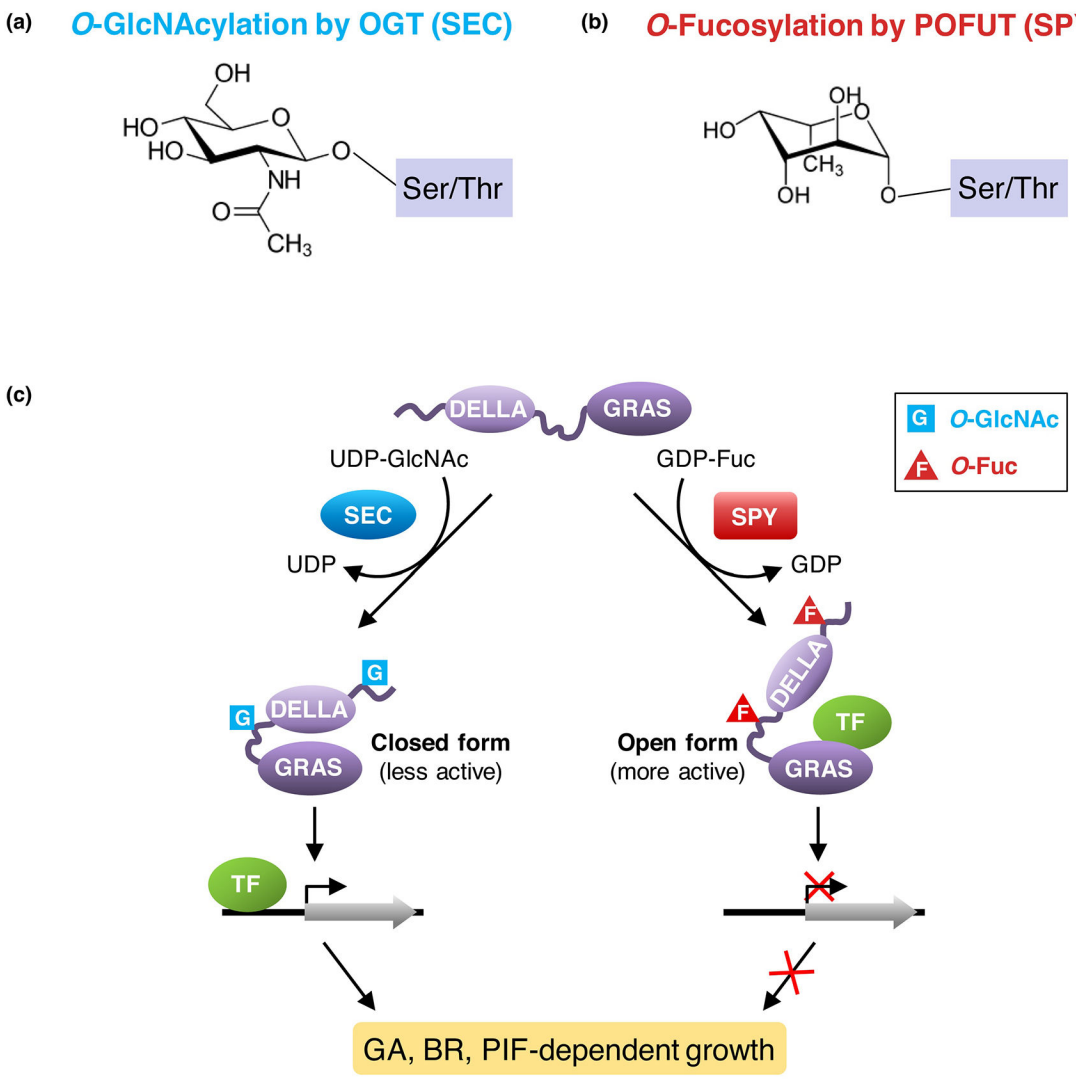

Figure 2. Model for the opposing roles of *O*-fucosylation and *O*-GlcNAcylation of DELLA in regulating plant growth.

(a) *O*-GlcNAcylation by OGT (SEC). (b) *O*-Fucosylation by POFUT (SPY). (c) The nuclear growth repressor DELLA proteins are activated by *O*-Fucosylation, and repressed by *O*-GlcNAcylation. Each DELLA protein contains an N-terminal DELLA domain and a C-terminal GRAS domain. *O*-Fucosylation (labeled as F) by SPY may induce the DELLA protein to an open conformation that is a more active growth repressor; this open form promotes binding of the GRAS domain to interacting transcription factors (e.g., BZR1 and PIFs), which leads to down-regulated expression of target genes of BZR1 and PIFs to restrict plant growth. In contrast, *O*-GlcNAcylation (labeled as G) by SEC may cause the DELLA protein to fold into a closed conformation that is less active because this form reduces its binding affinity to BZR1 and PIFs so that growth-related target genes can be activated. TF, DELLA-interacting transcription factor. The figure (c) was modified from Zentella et al. [14].

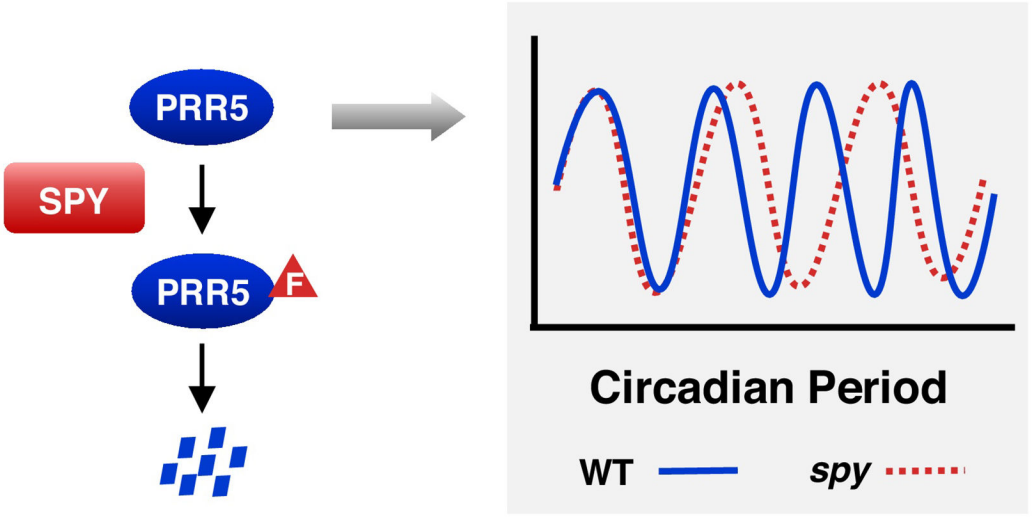

Figure 3. SPY regulates circadian period by inducing PRR5 degradation.

O-Fucosylation (labeled as F) of the transcription repressor PRR5 by SPY promotes PRR5 degradation. The *spy* mutant has a longer circadian period than WT.