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# Cross Talk between Gibberellin and Cytokinin Signaling Converges on SPINDLY

Cross talk in hormonal signaling pathways promises to be an important topic in cell and developmental biology in the 21st century. Before 1990, very little reference to cross talk appeared in the literature in any area of biology, and use of the term was restricted to the physical sciences, where it defines a disturbance that causes signals in adjacent electronic circuits to cross over each other. Since then, biologists have adopted the term cross talk to define points of regulatory interaction between two or more signal transduction pathways in living organisms. In both plant and animal biology, many of the major components of individual signal transduction pathways (as well as components of hormone biosynthesis pathways) have been identified. The challenge now is to determine the mechanisms of cross talk, or how all of these pathways interact and how they are coordinately regulated. In this issue of *The Plant Cell*, **Greenboim-Wainberg et al. (pages 92–102)** show that *SPINDLY* (*SPY*) functions both as a repressor of gibberellin (GA) responses and a positive regulator of cytokinin responses in Arabidopsis and present a model for SPY as a major coordinator of cross talk between these signaling pathways.

## THE DOUBLE LIFE OF SPY

The *SPY* locus in Arabidopsis was identified more than 10 years ago as a regulator of GA signal transduction. Five mutant alleles have been characterized: *spy1-3*, isolated from EMS-mutagenized populations based on resistance to the GA biosynthesis inhibitor paclobutrazol (Jacobsen and Olszewski, 1993), the T-DNA insertional mutant *spy-4* (Jacobsen et al., 1996), and *spy-5*, identified as a suppressor of the GA-insensitive mutation *gai* (Wilson and Somerville, 1995). All the of *spy* mutants exhibit recessively inherited phenotypic characteristics similar to those of wild-type plants treated with exogenous GA, suggesting that SPY functions as an inhibitor of GA responses. This was further confirmed by experiments showing that overexpression of *SPY* confers a phenotype consistent with reduced GA action (Swain et al., 2001). Apparent homologs of *SPY* have been cloned from barley (*HvSPY*; Robertson et al., 1998) and petunia (*PhSPY*; Izhaki et al., 2001).

One of the first indications that SPY might play a role in multiple pathways came from the detailed phenotypic analysis of *spy* mutants conducted by Swain et al. (2001). These authors showed that severe mutants exhibit several characteristics not seen in plants treated with GA, including abnormal leaf morphology and phyllotaxy. Greenboim-Wainberg et al. treated wild-type and *spy* mutant Arabidopsis seedlings to a number of phytohormones and found that *spy* mutants were partially resistant to cytokinin. Cytokinin-induced inhibition of root elongation and accumulation of anthocyanins in the wild-type seedlings were suppressed in *spy* mutants. The effects of the *spy* mutation on cytokinin responses could be mimicked by adding GA to cytokinin-treated wild-type seedlings, suggesting that GA inhibits cytokinin signaling. The effect of *spy* mutations on cytokinin responses therefore could possibly be an indirect effect of disrupting GA signaling. The authors use several lines of observation and reasoning to conclude that (1) SPY acts directly to promote cytokinin responses, (2) GA acts through SPY to suppress cytokinin responses, and (3) SPY regulates GA signaling and cytokinin responses via different mechanisms. The observations centered on analysis and comparisons of the *spy-4* and *spy-3* mutants, which represent strong and weak alleles, respectively, with respect to their GA responses (i.e., *spy-4* exhibits phenotypic alterations that resemble a strong increase in GA signaling, whereas *spy-3* is almost indistinguishable from the wild type), but display similar phenotypes with respect to their resistance to cytokinin.

## SPY MECHANISM OF ACTION AND THE IMPORTANCE OF *O*-GlcNAc MODIFICATION

*SPY* encodes a Ser and Thr *O*-linked *N*-acetylglucosamine transferase (OGT) that exhibits OGT activity in vitro (Thornton et al., 1999). OGT enzymes modify target proteins by transferring *N*-acetylglucosamine (GlcNAc) from UDP-GlcNAc to specific Ser and Thr residues via a covalent *O*-linkage. In SPY and all other OGTs, the catalytic domain is located in the C terminus, whereas the N terminus contains a series of tetratricopeptide repeat (TPR) motifs, which have been shown to mediate protein–protein interactions. Swain et al. (2002) showed that SPY is localized in the nucleus and cytosol and expressed throughout the plant. It is presumed that the protein functions as an OGT in complex with other proteins, but its in vivo targets and interacting partners are unknown. Yeast two-hybrid assays identified putative transcription factor MYB and NAC-like proteins as HvSPY-interacting partners in barley aleurone (Robertson, 2004) and GIGANTEA (GI; Tseng et al., 2004) as a SPY-interacting partner in Arabidopsis seedlings. *GI* was previously identified as a circadian clock–controlled gene that acts in the long day flowering pathway (Fowler et al., 1999). An interaction between SPY and GI might therefore help to explain some of the effects of GA on flowering time (Tseng et al., 2004).

*O*-GlcNAc modification is an abundant and reversible type of protein glycosylation

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found in all eukaryotes. It is distinct from other well-known forms of glycosylation that occur in the secretory pathway and instead takes place almost exclusively in the nucleus and cytoplasm (Hart, 1997). *O*-GlcNAc modification is essential in both plants and animals. Deletion of *OGT* in mouse is lethal (Shafi et al., 2000). Arabidopsis has two *OGT* genes, *SPY* and *SECRET AGENT* (*SEC*), and *spy sec* double mutants are embryo-lethal (Hartweck et al., 2002). *O*-GlcNAc modification has been likened to phosphorylation, in that it has diverse functions relating to the regulation of protein–protein interactions and/or protein function. Moreoever, all known *O*-GlcNAc modified proteins are also phosphoproteins, and *O*-GlcNAc modification occurs on the same Ser and Thr residues that are subject to phosphorylation. There is growing evidence that *O*-GlcNAc modification operates as a modulator of phosphoregulation affecting numerous regulatory proteins involved in signaling, transcription, and cytoskeletal functions (Kamemura and Hart, 2003).

The TPR domain confers specificity and versatility to OGT proteins. OGT proteins typically contain between 9 and 13 tandemly arrayed 34–amino acid TPR motifs that fold into a series of antiparallel α-helices, which form a recognition and docking site for target proteins (Das et al., 1998). Binding to certain substrates requires only a subset of the TPRs (Iyer and Hart, 2003), and different substrates might depend on different TPRs within the same protein. Thus, SPY (as well as SEC) has the potential to regulate various signaling pathways via interaction with different proteins through its TPR domains.

### A NEW MODEL FOR SPY FUNCTION

The results of Greenboim-Wainberg et al. fit a general model for SPY function that states that, in the absence of GA, SPY functions both to promote a subset of cytokinin responses and to inhibit the GA signaling pathway. GA accumulation (e.g., through the induction of biosynthesis or the addition of exogenous GA) is postulated to inhibit SPY function, which has the dual effect of repressing cytokinin responses and relieving the inhibition of GA signaling. It is important to note that SPY affects only a subset of the GA and cytokinin responses; both pathways appear to include downstream responses that are independent of SPY function.

A key question is whether both the repressor and positive regulator functions of SPY require OGT catalytic activity. For example, it is possible that some functions of SPY are dependent on the TPR domain but do not involve *O*-GlcNAc modification of target proteins. The observation that *spy-3* and *spy-4* exhibit significant differences in sensitivity to GA, but no apparent difference in their resistance to cytokinin, suggests that SPY might promote cytokinin responses and repress GA responses via distinct mechanisms.

The work of Greenboim-Wainberg et al. provides an important link between two of the major hormonal signaling pathways in higher plants. Future investigations into SPY function, and further knowledge of its interacting partners and the targets of *O*-GlcNAc modification, should reveal critical information about the cross talk between these signaling pathways.

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#### REFERENCES

**Das, A.K., Cohen, P.T.W., and Barford, D.** (1998). The structure of the tetratricopeptide repeats of protein phosphatase 5: Implications for TPR-mediated protein–protein interactions. EMBO J. **17,** 1192–1199.

**Fowler, S., Lee, K., Onouchi, H., Samach, A., Richardson, K., Morris, B., Coupland, G., and Putterill, J.** (1999). GIGANTEA: A circadian clock-controlled gene that regulates photoperiodic flowering in Arabidopsis and encodes a protein with several possible membrane spanning domains. EMBO J. **18,** 4679–4688.

**Greenboim-Wainberg, Y., Maymon, I., Borochov, R., Alvarez, J., Olszewski, N., Ori, N., Eshed, Y., and Weiss, D.** (2005). Cross talk between gibberellin and cytokinin: The Arabidopsis GA response inhibitor SPINDLY plays a positive role in cytokinin signaling. Plant Cell **17,** 92–102.

**Hart, G.W.** (1997). Dynamic *O*-linked glycosylation of nuclear and cytoskeletal proteins. Annu. Rev. Biochem. **66,** 315–335.

**Hartweck, L.M., Scott, C.L., and Olszewski, N.E.** (2002). Two *O*-linked *N*-acetylglucosamine transferase genes of *Arabidopsis thaliana* L. Heynh. have overlapping functions necessary for gamete and seed development. Genetics **161,** 1279–1291.

**Izhaki, A., Swain, S.M., Tseng, T., Borochov, A., Olszewski, N.E., and Weiss, D.** (2001). The role of SPY and SPY's TPR domains in the regulation of gibberellin action through the life cycle of *Petunia hybrida* plants. Plant J. **28,** 181–190.

**Jacobsen, S.E., Binkowski, K.A., and Olszewski, N.E.** (1996). SPINDLY, a tetratricopeptide repeat protein involved in gibberellin signal transduction in *Arabidopsis*. Proc. Natl. Acad. Sci. USA **93,** 9292–9296.

**Jacobsen, S.E., and Olszewski, N.E.** (1993). Mutations at the *SPINDLY* locus of Arabidopsis alter gibberellin signal transduction. Plant Cell **5,** 887–896.

**Kamemura, K., and Hart, G.W.** (2003). Dynamic interplay between O-glycosylation and O-phosphorylation of nucleoplasmic proteins: A new paradigm for metabolic control of signal transduction and transcription. Prog. Nucleic Acid Res. Mol. Biol. **73,** 107–136.

**Iyer, S.P.N., and Hart, G.W.** (2003). Roles of the tetratricopeptide repeat domain in *O*-GlnNAc transferase targeting and protein substrate specificity. J. Biol. Chem. **278,** 24608–24616.

**Robertson, M.** (2004). Two transcription factors are negative regulators of gibberellin responses in the HvSPY-signaling pathway in barley aleurone. Plant Physiol. **136,** 2747–2761.

**Robertson, M., Swain, S.M., Chandler, P.M., and Olszewski, N.E.** (1998). Identification of a negative regulator of gibberellin action, HvSPY, in barley. Plant Cell **10,** 995–1007.

**Shafi, R., Iyer, S.P., Ellies, L.G., O'Donnell, N., Marek, K.W., Chui, D., Hart, G.W., and Marth, J.D.** (2000). The *O*-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny. Proc. Natl. Acad. Sci. USA **97,** 5735–5739.

**Swain, S.M., Tseng, T.-S., and Olszewski, N.E.** (2001). Altered expression of *SPINDLY* affects gibberellin response and plant development. Plant Physiol. **126,** 1174–1185.

## IN THIS ISSUE

**Swain, S.M., Tseng, T.-S., Thornton, T.M., Gopalraj, M., and Olszewski, N.E.** (2002). SPINDLY is a nuclear-localized repressor of gibberellin signal transduction expressed throughout the plant. Plant Physiol. **129,** 605–615.

**Thornton, T., Kreppel, L., Hart, G., and Olszewski, N.E.** (1999). Genetic and biochemical analysis of *Arabidopsis* SPY. In Plant Biotechnology and in Vitro Biology in the 21st Century, A. Altman, M. Ziv, and S. Izhar, eds (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 445–448.

**Tseng, T.-S., Patrice, A., Salome, P.A., McClung, C.R., and Olszewski, N.E.** (2004). SPINDLY and GIGANTEA interact and act in *Arabidopsis thaliana* pathways involved in light responses, flowering, and rhythms in cotyledon movements. Plant Cell **16,** 1550–1563.

**Wilson, R.N., and Somerville, C.R.** (1995). Phenotypic suppression of the gibberellin-insensitive mutant (*gai*) of Arabidopsis. Plant Physiol. **108,** 495–502.