Signal transduction regulating meristem development in Arabidopsis

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Overview

Research support by DE-FG02-96ER20227 focused on the *CLV* loci and their regulation of organ formation at the Arabidopsis shoot meristem. Shoot meristem function is central to plant development as all of the above-ground organs and tissues of the plant are derived post-embryonically from the shoot meristem. At the shoot meristem, stem cells are maintained, and progeny cells undergo a switch toward differentiation and organ formation. The *CLV* loci, represented by three genes *CLV1*, *CLV2* and *CLV3* are key regulators of meristem development.

Each of the *CLV* loci encode a putative receptor-mediated signaling component. When this work began, virtually nothing was known about receptor-mediated signaling in plants. Thus, our goal was to both characterize these genes and the proteins they encode as regulators of meristem development, and to investigate how receptor-mediated signaling might function in plants.

Our work lead to several major publications that were significant contributions to understanding this system. The keys papers are described below.

Julie E. Stone, Amy E. Trotochaud, John C. Walker and Steven E. Clark (1998) "Control of meristem development by CLAVATA1 receptor kinase and kinase-associated protein phosphatase interactions," *Plant Physiology* **117**, 1217-1225.

The CLAVATA1 (CLV1) gene encodes a putative receptor kinase required for proper balance between cell proliferation and differentiation in Arabidopsis shoot and flower meristems. Impaired CLV1 signaling results in masses of undifferentiated cells at the shoot and flower meristems. Although many putative receptor kinases have been identified in plants, the mechanism of signal transduction mediated by plant receptor-like kinases is largely unknown. One potential effector of receptor kinase signaling is kinase-associated protein phosphatase (KAPP), a protein that binds to multiple plant receptor-like kinases in a phosphorylation-dependent manner. To examine a possible role for KAPP in CLV1-dependent plant development, the interaction of CLV1 and KAPP was investigated in vitro and in vivo. KAPP binds directly to atuophosphorylated CLV1 in vitro and co-immunoprecipitates with CLV1 in plant extracts dereived from meristematic tissue. reduction of *KAPP* transcript accumulation in an intermediate *clv1* mutant

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suppresses the mutant phenotype, and the degree of suppression is inversely correlated with *KAPP* mRNA levels. These data suggest that KAPP functions as a negative regulator of CLV1 signaling in plant development. This may represent a general model for the interaction of KAPP with receptor kinases.

Amy E. Trotochaud, Tong Hao, Guang Wu, Zhenbiao Yang and Steven E. Clark (1999) "The CLAVATA1 receptor-like kinase requires CLAVATA3 for its assembly into a signaling complex that includes KAPP and a Rho-related protein," *Plant Cell* **11**, 393-405.

The *CLAVATA1* (*CLV1*) and *CLAVATA3* (*CLV3*) genes are required to maintain the balance between cell proliferation and organ formation at the Arabidopsis shoot and flower meristems. *CLV1* encodes a receptor-like protein kinase. We have found CLV1 is present in two protein complexes in vivo. One is ~185 kD, and the other is ~450 kD. In each complex, CLV1 is part of a disulfide-like multimer of ~185 kD. The 450-kD complex contains the protein phosphatase KAPP, which is a negative regulator of CLV1 signaling, and a Rho GTPase-related protein. In clv1 and clv3 mutants, CLV1 is found primarily in the 185-kD complex. We propose that CLV1 is present as an inactive disulfide-linked heterodimer and that CLV3 functions to promote the assembly of the active 450-kD complex, which then relays signal transduction through a Rho GTPase.

Sangho Jeong, Amy E. Trotochaud and Steven E. Clark (1999) "The *CLAVATA2* gene encodes a receptor-like protein required for the stability of the CLAVATA1 receptor-like kinase," *Plant Cell* **11**, 1925-1933.

The *CLAVATA2* (*CLV2*) gene regulates both meristem and organ development in Arabidopsis. We isolated the *CLV2* gene and found that it encodes a receptor-like protein (RLP), with a presumed extracellular domain composed of leucine-rich repeats similar to those found in plant and animal receptors, but with a very short predicted cytoplasmic tail. RLPs lacking cytoplasmic signaling domains have not been previously shown to regulate development in plants. Our prior work has demonstrated that the CLV1 receptor-like kinase (RLK) is present as a disulfide-linked multimer in vivo. we report that CLV2 is required for the normal accumulation of CLV1 protein and its assembly into protein complexes, indicating CLV2 may form a heterodimer with CLV1 to transduce extracellular signals. Sequence analysis suggests that the charged residue in the predicted transmembrane domain of CLV2 may be a common feature of plant RLPs and RLKs. In addition, the chromosomal region in which *CLV2* is located contains an extremely high rate of polymorphism, with 50 nucleotide and 15 amino acid differences between Landsberg *erecta* and Columbia ecotypes within the *CLV2* coding sequence.

Lita P. Yu, Ephraim J. Simon and Steven E. Clark (2000) "*POLTERGEIST* functions to regulate meristem development downstream of the *CLAVATA* loci," *Development* **127**, 1661-1670.

Mutations at the *CLAVATA* loci (*CLV1*, *CLV2* and *CLV3*) result in the accumulation of undifferentiated cells at the shoot and floral meristems. We have isolated three mutant

alleles of a novel locus, *POLTERGEIST* (*POL*), as supressors of *clv1*, *clv2* and *clv3* phenotypes. All *pol* mutants were nearly indistinguishable from wild-type plants; however, *pol* mutants provided recessive, partial suppression of meristem defects in strong *clv1* and *clv3* mutants, and nearly complete suppression of weak *clv1* mutants. *pol* mutations partially suppress *clv2* floral and pedicel defects in a dominant fashion, and almost completely suppressed *clv2* phenotypes in a recessive manner. These observations, along with dominant interactions observed between *pol* and *wuschel* (*wus*) mutations, indicate that *POL* functions as a critical regulator of meristem development downstream of the *CLV* loci and redundantly with *WUS*. Consistent with this, *pol* mutations do not suppress *clv3* phenotypes by altering *CLV1* receptor activation.