G211

Localization and Functional Analysis of the Glutamate Receptor from Small Radish

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The ionotropic glutamate receptors (iGluRs) function as glutamate-activated ion channels in rapid synaptic transmission in animals. Calcium entry through glutamate receptor channels play important roles in development and in forms of synaptic plasticity. Recently it was proposed that plant GLRs function as NSCCs, but there havebeen no direct evidences for glutamate gating and localization of these channels. Accordingly, we have isolated and characterized a cDNA encoding glutamate receptor from small radish (RsGluR). To determine the subcellular localization of RsGluR, GUS-GFP was fused with the C-terminus of RsGluR (RsGluR::GUS-GFP). The expression of RsGluR::GUS-GFP was ubiquitous on the plasmamembrane of seedlings. We also investigated the glutamate gating of RsGluR using the Fluo-4/AM (Ca^{2+} indicator) in Arabidopsis transformed with RsGluR cDNA. Transgenic Arabidopsis lines demonstrate increased permeability to Ca^{2+} only in the presence of glutamate. These results support the function of RsGluR as a glutamate gated Ca^{2+} permeable channel, the first direct functional evidence for plant glutamate receptor.

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Functional Characterization of a Floral Pathway Integrator SOC1: A Study for Identifying Interacting Partners and Characterizing the Functional Domains

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SOC1 is a MADS box protein functioning as a floral pathway integrator in Arabidopsis. Considering the previous reports, MADS box proteins are composed of four functional domains (MADS, I, K, and C) and usually function by forming dimers or tetramers. To characterize the functional domain of SOC1, domain swapping was done between SOC1 and FLC. FLC is also a MADS box protein, which functions as a negative regulator of flowering. According to our results, plants overexpressing the chimeric proteins with the SOC1 MADS domain showed early flowering. However, none of the other transgenic lines showed late flowering phenotype as severe as *35S::FLC*. An interesting phenotype was also observed in transgenic plants overexpressing chimeric proteins composed of FLC MADS and SOC1 IKC domains. These results indicate that each domain has different functions and that the interaction of each domain with other proteins might be important. Accordingly, we used Yeast Two Hybrid systems to analyze the interacting partners with SOC1 and FLC. In addition, the interaction is also tested with the chimeric proteins. So far, our results propose a strong evidence of SOC1 functioning as a complex in vivo. Thus, we adapted the Tandem Affinity Purification (TAP) method to purify the SOC1 complex as a whole from the wild type plant. The progress so far on using TAP is also discussed.

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Characterization and Cloning of *SUF* (*Suppressor of FRI*) Mutants that Regulate Flowering Time in Arabidopsis

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Flowering of Arabidopsis is promoted by several interacting genetic pathways; photoperiod, vernalization and autonomous pathways. In the autonomous pathway, *FLOWERING LOCUS C* (*FLC*) is known as a central molecule, which acts as a floral repressor. *FLC*is positively regulated by *FRI*, while it is negatively regulated by *LD*, *FVE*, *FCA* and vernalization treatment. .By fast neutron mutagenesis of *FRI*-Col, a very late flowering line, we isolated mutants that flower as early as Columbia ecotype. By genetic complementation with *fri* and *flc*, 26 early flowering mutants were shown to be novel. The mutants were named as *suf* (*suppressor of FRI*). Our physiological data showed that the *sufs* had various sensitivity to photoperiods (short day vs. long day) and vernalization (4?, 8 weeks). Northern analyses showed that the expression of *FLC* in *suf3* was slightly decreased and was not detected in *suf4* and *suf5*. But *ALG20* and *FT* were highly expressed as in Col in them. These results suggest that *SUF3* acts downstream of *FLC* while *SUF4* and *SUF5* act upstream of *FLC* in the autonomous pathway. The Cloning of *SUFs* are in pregress.

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Analysis of the Flowering Time Regulator, *fsu2* (FRI Suppressor 2)

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The late-flowering trait of Arabidopsis winter annual ecotype is conferred mainly by two genes: *FRIGIDA (FRI)* and *FLOWERING LOCUS C (FLC)* . To further elucidate the genetic control of flowering, we have screened *FRI* suppressor mutant by activation-tagging mutagenesis. In this sdtudy, one-early flowering mutant, *fsu2* (*FRI* suppressor 2), was isolated. The *fsu2* was dominant and homozygous *fsu2* mutant showed slightly vernalization sensitivity. T-DNA was inserted in the second intron of novel MADS box gene (*FSU2A*). *FSU2A* showed highest similarity to *FSU2B*, which is located next to *FSU2A*. The amino acid sequences of FSU2A and FSU2B showed high similarity to AGL20. Both *FSU2A* and *FSU2B* genes are overexpressed in *fsu2* mutant. Loss and reduction of *FSU2B* expression by RNAi result in late- flowering , whereas FSU2A enhancer line (FSU2A-EN) FSU2B enhancer line (FSU2B-EN) double mutant produces earlly-flowering. Therefore, these results suggest that early flowering phenotype of *fsu2* mutant results from the interaction between partial FSU2A and full FSU2B.