

Two SERK Receptor-Like Kinases Interact with EMS1 to Control Anther Cell Fate Determination¹[OPEN]

Zhiyong Li, Yao Wang, Jian Huang, Nagib Ahsan², Gabriel Biener, Joel Paprocki, Jay J. Thelen, Valerica Raicu, and Dazhong Zhao*

Department of Biological Sciences (Z.L., Y.W., J.H., V.R., D.Z.) and Department of Physics (G.B., J.P., V.R.), University of Wisconsin, Milwaukee, Wisconsin 53211; and Department of Biochemistry, University of Missouri, Columbia, Missouri 65211 (N.A., J.J.T.)

ORCID IDs: 0000-0002-2151-5535 (Z.L.); 0000-0001-9167-1421 (J.H.); 0000-0001-5995-1562 (J.J.T.); 0000-0002-3427-8516 (V.R.); 0000-0002-2045-0717 (D.Z.).

Cell signaling pathways mediated by leucine-rich repeat receptor-like kinases (LRR-RLKs) are essential for plant growth, development, and defense. The EMS1 (EXCESS MICROSPOROCTES1) LRR-RLK and its small protein ligand TPD1 (TAPETUM DETERMINANT1) play a fundamental role in somatic and reproductive cell differentiation during early anther development in *Arabidopsis* (*Arabidopsis thaliana*). However, it is unclear whether other cell surface molecules serve as coregulators of EMS1. Here, we show that SERK1 (SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE1) and SERK2 LRR-RLKs act redundantly as coregulatory and physical partners of EMS1. The *SERK1/2* genes function in the same genetic pathway as *EMS1* in anther development. Bimolecular fluorescence complementation, Förster resonance energy transfer, and coimmunoprecipitation approaches revealed that SERK1 interacted biochemically with EMS1. Transphosphorylation of EMS1 by SERK1 enhances EMS1 kinase activity. Among 12 *in vitro* autophosphorylation and transphosphorylation sites identified by tandem mass spectrometry, seven of them were found to be critical for EMS1 autophosphorylation activity. Furthermore, complementation test results suggest that phosphorylation of EMS1 is required for its function in anther development. Collectively, these data provide genetic and biochemical evidence of the interaction and phosphorylation between SERK1/2 and EMS1 in anther development.

Plants and animals share similar mechanisms for cell-cell and cell-environment communications. Signaling in multicellular organisms relies predominantly on receptor kinases, and this is particularly true for plants. In *Arabidopsis* (*Arabidopsis thaliana*), more than 600 genes encode receptor-like kinases (RLKs), with 223 Leu-rich repeat (LRR) RLKs forming the largest family of RLKs

(McCarty and Chory, 2000; Shiu et al., 2004; Torii, 2004; Zhao, 2009). Extensive research has shown that LRR-RLKs are involved in a wide range of plant growth, developmental, and physiological processes as well as defense responses, including the regulation of shoot and root meristem sizes, cell fate determination and patterning, steroid hormone signaling, vascular patterning, organ size and shape regulation, organ abscission, pollen tube reception, defense responses, plant transpiration, nodulation, and nitrogen acquisition (Torii, 2004; Zhao, 2009; Gursansky et al., 2016; Ma et al., 2016; Shinohara et al., 2016; Takeuchi and Higashiyama, 2016; Wang et al., 2016). So far, only a few coreceptors have been identified for the many functionally characterized LRR-RLKs.

In flowering plants, successful sexual reproduction depends on the specification of distinct types of somatic and reproductive cells that yield male and female gametophytes. The anther, where male gametophytes (pollen) are produced, typically has four lobes (microsporangia; Goldberg et al., 1993; Scott et al., 2004; Zhao, 2009; Feng et al., 2013; Walbot and Egger, 2016). Within each of these lobes in a mature anther, the central reproductive microsporocytes (or pollen mother cells) are surrounded by four concentrically organized somatic cell layers: epidermis, endothecium, middle layer, and tapetum (Zhao et al., 2002; Zhao, 2009). Microsporocytes give rise to pollen via meiosis, while somatic cells, particularly the tapetum, are required for the normal development

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² Present address: Department of Biology and Medicine, Brown University, and Center for Cancer Research and Development, Proteomics Core Facility, Rhode Island Hospital, Providence, RI 02903.

* Address correspondence to dzhao@uwm.edu.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Dazhong Zhao (dzhao@uwm.edu).

D.Z. conceived and designed the experiments; D.Z., J.J.T., and V.R. supervised the experiments; Z.L., Y.W., J.H., N.A., G.B., and J.P. performed most of the experiments; all authors analyzed data; D.Z. and V.R. wrote the article with contributions of all coauthors.

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