Direct Phosphorylation and Activation of MPK5 by Membrane-associated CPK18 in Rice

PAG XXIII, Jan. 13, 2015

Kabin Xie, Jianping Chen, Qin Wang, Yinong Yang
Pennsylvania State University

Mitogen-Activated Protein Kinases

- Highly conserved in eukaryotes
- Mediate cellular responses to a diversity of endogenous and exogenous signals and stimuli
- Important for plant growth and development as well as disease resistance and abiotic stress tolerance

Classical MAPK Cascade

- MAPK Kinase (MKK)
- MAP Kinase (MPK)
- T/E/D-Y
- S/T-P
- Substrate

OsMPK5 Inversely Mediates Rice Disease Resistance and Abiotic Stress Tolerance

- Drought
- Salinity
- Cold
- Wounding
- Pathogen

- Abscisic Acid
- Ethylene
- Abiotic Stress Tolerance
- Disease Resistance


Plant Stress Signal Transduction Mediated by Protein Phosphorylation

- Sensor/receptors
- Secondary messengers
- Protein kinases
- Transcriptional factors
- Gene expression
- Responses

Rice MAP Kinase Family

- A total of 17 MPK genes found in rice genome
- Over a half are associated with biotic and abiotic responses

- MPK2: biotic stress, cell death
- MPK3: ABA, ET, biotic and abiotic stress (AtMPK3 orthologue)
- MPK5: biotic stress
- MPK6: biotic stress
- MPK7: JA, biotic and abiotic stress
- MPK13: ET, SA, biotic stress
- MPK17: ET, ABA, biotic and abiotic stress

(Reyna and Yang 2006; Rohila and Yang 2007; Shen et al 2010; Mitsuko Kishi-Kabosh et al 2010)

Identification of OsMPK5 Substrates by Phosphorylation Screening

- 4 protein kinases (CPK18)
- 1 protein phosphatase
- 1 protease
- 3 transcription factors
- 1 RNA-binding protein
- 1 cold shock protein
- 1 ion channel

* Associated with biotic and/or abiotic stress response.
Harper et al. 2004

V, N-terminal variable domain
K, Kinase domain
J, Junction/autoinhibitor domain,
C, calmodulin domain (Ca\(^{2+}\) binding domain)

Rice CDPK family
- Four phylogenetic groups.
- A total of 29 CDPKs in rice.
- CPK18 and CPK4 belong to Group IV CDPKs in rice.

CPK18

Al, Arabidopsis thaliana
Os, Oryza sativa

Direct Interaction and Phosphorylation Between CPK18 and MPK5 In Vitro

In vitro kinase assay In vitro GST pull down

CPK18DA (D178A substitution, barely detectable autophosphorylation)
MPK5KR (K65R substitution, no detectable autophosphorylation)

Xie et al. (2014) Plant Cell 26: 3077-3089

In vivo Interaction Between CPK18 and MPK5

CPK18 is a plasma membrane-associated protein kinase.
CPK18-MPK5 complex is predominantly localized in plasma membrane.

CDPK Phosphorylates MPK5 In Vivo

In gel kinase assay reveals that CPK18 is a major kinase of MPK5

Phosphorylation Between Specific Subgroups of Plant CDPKs and MAPKs

Group IV CDPKs phosphorylate Group A/B MAPKs

CDPK, ~60kDa
MKK, ~40 kDa
EGTA: Ca\(^{2+}\) chelator.
CPK18 Phosphorylates MPK5 at T14 and T32

Mimicking T32 Phosphorylation at MPK5 Enhances Its Kinase Activity

CPK18 Positively Regulates MPK5 Activity In Vivo

In gel kinase assay of total protein

In gel kinase assay of IP-MPK5 activity

CPK18 – MPK5 Pathway Negatively Regulates Rice Resistance to *Magnaporthe oryzae*

CPK18 – MPK5 Pathway Negatively Regulates Defense Gene Expression in Rice

Multiplex Targeted Mutagenesis of 4 MPKs in Stable Transgenic Rice Plants

Current Lab Members:
Dr. Yinong Yang
Dr. Kabin Xie         Bastian Minkenberg
Dr. Xiangling Shen   Yueying Chen
Qin Wang              Xiaoyi Guo

Huck Institutes of Life Sciences
Penn State University