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授与した学位	博　士
専攻分野の名称	農　学
学位授与番号	博甲第５２３６号
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学位授与の要件	環境生命科学研究科　農生命科学専攻 （学位規則第５条第１項該当）
学位論文の題目	Microbe-associated molecular pattern signaling in Arabidopsis guard cells （シロイヌナズナ孔辺細胞における microbe-associated molecular pattern 信号伝達）
論文審査委員	教授　村田　芳行　　教授　中村　宜督　　教授　木村　吉伸

学位論文内容の要旨

Stomata, formed by pairs of guard cells in the epidermis of terrestrial plants, regulate gas exchange including transpiration and CO_2 entry for photosynthesis, thus playing a critical role in plant growth. Recent studies reveal that stomata are exploited by microbes as an entry route. Microbe-associated molecular patterns (MAMPs) are molecular signatures that are highly conserved in whole classes of microbes but are absent from the host, such as chitin for fungi and flagellin for bacteria. So far, research has revealed that guard cells perceive MAMPs from microbes leading to the stomatal closure. However, the mechanism of MAMP-induced stomatal closure remains largely unknown. The present study is set to further elucidate the mechanism of MAMP-induced stomatal closure. Yeast elicitor (YEL) extracted by ethanol precipitation mainly contains fungal cell wall fraction and has been widely used as MAMPs to induce plant immune responses. In the present study, I used YEL as a MAMP material.

Activation of S-type anion channels is essential for stomatal closure, while deactivation of inward-rectifying K^+ channels (K_{in} channels) is preferred in stomatal closure. Cytosolic Ca^{2+} has long been recognized as a critical second messenger in guard cell signaling. Elevation of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) is important to activation of S-type anion channels and suppression of K_{in} channel activity induced by various stimuli. Influx of Ca^{2+} is carried by nonselective Ca^{2+}-permeable cation channels (I_{Ca} channels) that can be activated by H_2O_2 and contributes to $[Ca^{2+}]_{cyt}$ elevation. In the present study, the effect of YEL on I_{Ca} channels, K_{in} channels and S-type anion channels in Arabidopsis guard cell protoplasts was investigated. YEL induced activation of I_{Ca} channels that were permeable to Ca^{2+}. YEL suppressed K_{in} channel currents but had no effect on K_{in} channel gating properties. YEL induced activation of S-type anion channel currents when the $[Ca^{2+}]_{cyt}$ was elevated to 2 μM. These results suggest that YEL activates I_{Ca} channels and S-type anion channels and suppresses K_{in} channel activity in Arabidopsis guard cells.

A Ca^{2+}-dependent protein kinase, CPK6, is activated by Ca^{2+} and is essential to activation of S-type channels and stomatal closure induced by abscisic acid (ABA) and methyl jasmonate. In the present study, I investigated the role of CPK6 in YEL-induced stomatal closure in Arabidopsis. Disruption of *CPK6* gene impaired YEL-induced stomatal closure, and H_2O_2 accumulation, I_{Ca} channel activation, $[Ca^{2+}]_{cyt}$ elevations, S-type anion channel activation and suppression of K_{in} channel activity in guard cells. These results suggest that CPK6 positively functions in YEL-induced stomatal closure in Arabidopsis, and is a convergent point of signaling pathways for stomatal closure in response to abiotic and biotic stress.

Ca^{2+}-independent pathways regulate ABA-induced stomatal closure. A Ca^{2+}-independent protein kinase, Open Stomata 1 (OST1), is involved in stomatal closure induced by various stimuli. In the present study, I investigated the role of OST1 in YEL-induced stomatal closure in Arabidopsis using a knock-out mutant, *ost1-3*, and a kinase-deficient mutant, *ost1-2*. YEL did not induce stomatal closure or activation of guard cell S-type anion channels in the *ost1* mutants unlike in wild-type plants. However, YEL did not increase OST1 kinase activity in wild-type guard cells. In the *ost1* mutants like in the wild type, YEL induced H_2O_2 accumulation, activation of I_{Ca} channels and transient elevations in $[Ca^{2+}]_{cyt}$ in guard cells. These results reveal that OST1 kinase is essential for stomatal closure and activation of S-type anion channels induced by YEL and that OST1 is not involved in H_2O_2 accumulation, I_{Ca} channel activation, or $[Ca^{2+}]_{cyt}$ elevations in guard cells induced by YEL, thus providing a *in vivo* evidence that OST1 kinase mediates a Ca^{2+}-independent pathway in stomatal closure induced by YEL.

Considering that YEL contains multiple MAMP components, CPK6 as well as OST1 is likely to commonly function as essential components of multiple MAMP signalings in Arabidopsis guard cells.

論文審査結果の要旨

気孔開閉は、陸上植物にとって重要な生理的現象であり、病原菌感染にも深く関わる。本論文は、病原菌構成成分である病原微生物関連分子パターン（Microbe-associated molecular pattern: MAMP）が誘導する気孔閉口における信号伝達経路を明らかにしようとしたものである。

初めに、MAMPが誘導する気孔閉口は、カルシウムチャネルやS型アニオンチャネルの活性化ならびに内向き整流性カリウムチャネルの不活性化を伴うことを明らかにした。

次に、孔辺細胞MAMP信号伝達経路において、カルシウム依存性タンパク質キナーゼであるCPK6が、活性酸素種（ROS）産生やS型アニオンチャネルの活性化を制御していることを明らかにした。

また、孔辺細胞MAMP信号伝達経路において、カルシウム非依存性タンパク質キナーゼであるOST1は、ROS産生や細胞質カルシウム濃度上昇には関与せずに、下流のS型アニオンチャネルの活性を制御していることを明らかにした。

以上の結果から、MAMPは、孔辺細胞CPK6やOST1を介して、気孔閉口を誘導することが明らかになった。

さらに、本研究内容は、学術的な価値のみならず、気孔運動に着目した生産制御のための技術の基礎となるものである。従って、本審査委員会は本論文が博士（農学）の学位論文に値すると判断した。