学位論文内容の要旨

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学位論文題名

The study of salicylic acid-mediated defense responses by a tobacco calmodulin-like protein

（タバコカルモジュリン様タンパク質により誘導されるサリチル酸を介した防御応答に関する研究）

Plant calmodulin (CaMs) and calmodulin-like protein (CMLs) are calcium ion (Ca^{2+}) sensors that play important roles in development and stress responses. An increase in the Ca^{2+} concentration in the cytoplasm is one of the earliest events following exposure to environmental stresses and Ca^{2+} is a crucial secondary messenger in the perception of these stresses. In plants, CaMs and CMLs constitute a relatively large family of Ca^{2+} sensor genes. CaMs and CMLs bind a number of endogenous factors but have no obvious functional domains except for 1−7 EF-hand motifs for binding Ca^{2+}, and thus are considered to transduce Ca^{2+} signals by modifying the activity or conformation of their binding endogenous proteins. One of the tobacco CMLs, rgs-CaM, uniquely binds to exogenous proteins, diverse viral RNA silencing suppressors (RSSs), presumably via affinity to their positively charged dsRNA-binding sites. rgs-CaM has previously been shown to be antiviral protein; that is, it directs degradation of viral RSSs via autophagy, resulting in reinforcement of antiviral RNA silencing.

In this study, rgs-CaM was shown to function as an immune receptor to recognize virus infection and induce defense reactions. Defense reactions and salicylic acid (SA) signaling induced by overexpressed or ectopically expressed rgs-CaM was examined. Then, how tobacco plants induce SA signaling via recognition of virus infection by rgs-CaM was studied.

1. Overexpressed and ectopically expressed rgs-CaM induces defense reactions and SA signaling

Among transgenic tobacco plants that constitutively overexpressed the *rgs-CaM* gene under the control of the cauliflower mosaic virus 35S promoter, two lines showed

dwarfing, deformation, and necrotic parts on their leaves. These phenotypes were similar to those of lesion mimic mutants that involve hypersensitive response–like programmed cell death, which is accompanied by induction of defense signaling components, including SA. In the transgenic plants showing these phenotypes, mRNA of the pathogenesis-related protein 1a gene (*PR1a*), an indicator of activation of SA signaling, was induced in the leaves. The results indicate the possibility that the overexpressed rgs-CaM can induce cell death and SA signaling, this possibility was confirmed using the PVX vector expressing rgs-CaM. Infection with this recombinant vector caused necrotic spots and induced *PR1a*, whereas infection with the empty PVX vector did not. Taken together, these data suggest that overexpressed and ectopically expressed rgs-CaM induced cell death and SA signaling.

2. rgs-CaM induces salicylic acid signaling via perception of both Ca^{2+} and viral RSS

When cucumber mosaic virus (CMV) was inoculated into wild-type and rgs-CaM–knockdown tobacco plants, *PR1a* was strongly induced in inoculated leaves of wild-type plants but not in those of rgs-CaM–knockdown plants, suggested the possibility that endogenous rgs-CaM is also involved in induction of SA signaling. Mechanical inoculation caused wounding and thus Ca^{2+} influx on the epidermal cells of inoculated leaves. CMV expresses the 2b protein, which is an RSS that interacts with rgs-CaM, in infected cells. Thus, rgs-CaM was hypothesized to perceive Ca^{2+} and viral RSSs for induction of SA signaling. As expected, *PR1a* was expressed in leaves of transgenic tobacco plants expressing viral RSS, 2b or clover yellow vein virus HC-Pro 24 h after wounding or induction of Ca^{2+} influx with an ionophore A23187. This *PR1a* induction was attenuated by silencing of the rgs-CaM expression. These results suggest that tobacco plants induced SA signaling in response to CMV infection via perception of Ca^{2+} and 2b by rgs-CaM. SA signaling is one of the major antiviral defense signaling. rgs-CaM–knockdown tobacco plants were more susceptible to CMV than wild-type tobacco plants. rgs-CaM seems to effectively inhibit CMV infection through 2b recognition and degradation by rgs-CaM, leading to reinforcement of antiviral RNA silencing and other SA–mediated antiviral responses.