Abstract

In pathogen-infected or wounded plants, Ca^{2+} plays a pivotal role in triggering defense responses including the rapid generation of reactive oxygen species (ROS) and nitric oxide (NO), activation of protein kinases and phosphatases, and induction of defense-related compounds such as phytoalexins and pathogenesis-related (PR) proteins. I isolated 13 novel calmodulin (CaM) genes, *NtCaM1* to *13*, from tobacco plant (*Nicotiana tabacum* cv. Samsun NN), whose encoded proteins were categorized into three different groups; group I (NtCaM1/2), II (NtCaM3/4/5/6/7/8/11/12 and 9/10) and III (NtCaM13). Their expression profile was analyzed in response to pathogen infection and wounding using specific DNA probes for individual CaM genes and antigen-affinity-purified specific antibodies for respective types of CaM proteins. Hypersensitive reaction (HR) in tobacco mosaic virus (TMV)-infected tobacco leaves accompanied a predominant accumulation of *NtCaM1*, *2*, *13* transcripts and NtCaM13-type protein, which is a possible ortholog of soybean defense-involved CaM, SCaM-4, preceding induction of *PR-1* and *-3* defense genes. In contrast, wounding induced accumulation of *NtCaM1*, *2*, *3*, and *4* transcripts within 30 min, and NtCaM1-type protein accumulated transiently after wounding. NtCaM13-type protein, which was found at a low level in healthy leaves, decreased instantly after wounding. The treatment with a proteasome inhibitor, lactacystin enhanced wound-induced accumulation of NtCaM1-type protein and inhibited wound-induced decrease of NtCaM13-type protein, suggesting that proteasome activity is involved in the degradation of these CaMs. Thus, these results indicate that levels of individual CaM proteins are differentially regulated both transcriptionally and post-transcriptionally in tobacco plants that are exposed to stresses such as pathogen-induced hypersensitive cell death and wounding.

Furthermore, I characterized the down-stream responses of these CaM isoforms. *In vitro* target enzyme assays revealed that wound-inducible NtCaM1 and NtCaM3 are potent activators for pea NAD^+ kinase which is believed to be involved in the production of ROS, whereas NtCaM13 is not. A CaM-dependent protein phosphatase, bovine calcineurin (CaN), was activated strongly by NtCaM3 and moderately by NtCaM1 and NtCaM13. In contrast, rat NO synthase (NOS) was most activated by the HR-dominant CaM, NtCaM13. Moreover, transgenic tobacco plants overproducing the NtCaM13 protein constitutively expressed PR genes, confirming the involvement of NtCaM13 in HR. Overexpression of *NtCaM1* gene led to an accumulation of the transcript for a wound-induced MAP kinase gene, *wipk*, without wounding, while suppression of *NtCaM1* resulted in little accumulation of the *wipk* transcript during HR, suggesting that NtCaM1 positively regulates *wipk* gene expression. Consequently, distinct CaM isoforms likely share roles after pathogen attack and wounding in tobacco plants.