Induction mechanism of hypersensitive cell death in rice mediated by transcription factor, OsNAC

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The hypersensitive response (HR) cell death is a common feature of plant immune responses and a type of programmed cell death. We recently reported that plant-specific transcription factor OsNAC4 is a positive regulator of HR cell death in rice. To clarify the induction mechanism of HR cell death by OsNAC4, the subcellular localization of OsNAC4 during HR cell death was examined. Phosphorylation-dependent translocation of OsNAC4 into nucleus was observed during HR cell death. Site-directed mutagenesis of OsNAC4 showed that five Ser residues within NAC domain are phosphorylated and the translocation of OsNAC4 into nuclei was regulated by the phosphorylation. To clarify the mechanism of transcriptional regulation by OsNAC4, yeast two-hybrid analysis using OsNAC4 as bait was performed. The analysis showed that OsNAC4 specifically interacted with OsNAC3, which is a member of OsNAC3 subfamily. The overexpression of OsNAC3 caused clear HR cell death accompanied with DNA fragmentation. No HR cell death was induced in OsNAC3 knockdown mutants when avirulent N1141 strain of *Acidovorax avenae* inoculated to the mutant cells. OsNAC3 was translocated into nuclei during HR cell death like OsNAC4. BiFC analysis revealed that OsNAC3 was interacted with OsNAC4 in nuclei. When OsNAC3 and OsNAC4 were co-introduced into rice cells, very strong HR cell death was induced. Moreover, the *OsHSP90* and *IREN* that were regulated by OsNAC4 were not induced in OsNAC3 knockdown mutants despite of the avirulent strain inoculation. These data suggest that protein complex which was made with OsNAC4 and OsNAC3 regulate the transcription of these genes.

We previously reported that transcription of *IREN* which encodes a novel EF-hand containing plant nuclease is controlled by OsNAC4, a key positive regulator of HR cell death. IREN contains two nuclear localization signals, and analysis of IREN-GFP localization in transiently transformed rice protoplasts revealed initial localization in nuclei. Transient overexpression of IREN in rice protoplasts also led to rapid DNA degradation as detected by TUNEL. Maximum DNA degradation associated with the recombinant IREN was observed in the presence of Ca^{2+} and Mg^{2+} or Ca^{2+} and Mn^{2+}. Interestingly, DNA degradation mediated by the recombinant IREN was completely abolished by Zn^{2+}, even when Ca^{2+}, Mg^{2+}, or Mn^{2+} were present in the reaction buffer. Based on these data, we conclude that IREN functions in the degradation of nuclear DNA

during HR cell death. Moreover, although DNA degradation mediated by IREN retarded the cell growth, transient over expression of *IREN* in *N. benthamiana* or cultured rice cells did not cause HR cell death. These data suggest that the DNA degradation events are not the direct trigger of HR cell death but are rather downstream phenomena that are parts of the HR cell death.