

Structural mechanism of NAD⁺ cleavage by plant TIR domain**Wen Song^{1,2}, Dongli Yu^{1,2} and Jijie Chai^{1,2}**¹*Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany*²*Institute of Biochemistry, University of Cologne, 50674 Cologne, Germany.
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Plant nucleotide-binding leucine-rich repeat (NLR) immune receptors recognize pathogen effectors to trigger cell death and confer disease resistance [1]. The Toll/interleukin-1 receptor (TIR) domains of plant NLRs can hydrolyze nicotinamide adenine dinucleotide in its oxidized form (NAD⁺), which is required for NLR-mediated immune signalling [2, 3]. The Cryo-EM structures of the RPP1 and Roq1 resistosomes show that formation of two asymmetric dimers of TIR domains is critical for the NADase activity [4, 5]. However, the structural mechanism underlying TIR-catalyzed NAD⁺ cleavage remains unknown. Here, we report a crystal structure of RPP1-TIR in complex with NAD⁺. The TIR domain forms a tetramer in an asymmetric unit, which is nearly identical with that seen in the RPP1 resistosome. The NAD⁺ is bound to the catalytic center between the asymmetric head-to-tail TIR homodimers, with the adenosine group contacting one TIR monomer (TIRb) and the phosphate groups and the nicotinamide ribose contacting the other TIR (TIRa). The nicotinamide-ribose bond of NAD⁺ has been cleaved, and the free nicotinamide stacks against the adenosine group. The carboxylate oxygen of the catalytic Glu158 interacts with the C-2 and C-3 hydroxyl groups of the nicotinamide ribose, and the interactions are highly conserved in the cADPR-bound ADP-ribosyl cyclase CD38. Our study reveals NAD⁺ recognition mechanism of a plant TIR domain and provides insight into NAD⁺ hydrolysis catalyzed by the TIR protein.

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