Structures of a DYW domain shed first light on a unique plant RNA editing regulation principle

Mizuki Takenaka[1], Tatjana Barthel[2], Sachi Takenaka[1], Brody Frink[1], Sascha Haag[3], Daniil Verbitskiy[3], Bastian Oldenkott[4], Mareike Schallenberg-Rüdinger[4], Christian Feiler[2], Manfred S. Weiss[2], Gottfried J. Palm[5]; Gert Weber[2]

[1]Department of Botany, Graduate School of Science, Kyoto University, Kyoto, Japan; [2]Helmholtz-Zentrum-Berlin (HZB), Berlin, Germany; [3]Molekulare Botanik, Universität Ulm, Germany; [4]IZMB – Institut für Zelluläre und Molekulare Botanik, Abt. Molekulare Evolution, University of Bonn, Bonn, Germany [5]University of Greifswald, Molecular Structural Biology, Greifswald, Germany

As part of RNA editosomal protein complexes, pentatricopeptide repeat (PPR) proteins with a C-terminal DYW domain have been characterized as site-specific factors for C to U RNA editing in plant mitochondria and plastids [1-2]. While substrate recognition is conferred by their repetitive PPR tract, the exact role of the DYW domain had not been clarified. The DYW domain shares a low sequence conservation with known cytidine deaminase structures (from 5 to 19% residue identities). Lastly, missing structural information had left the exact function and catalytic properties of DYW domains within the RNA editosome open [3]. We present functional data of an *Arabidopsis thaliana* DYW domain and structures in an inactive ground state and a catalytically activated conformation. DYW domains harbor a cytidine deaminase fold and a C-terminal DYW motif, with catalytic and structural Zn atoms, resp. The deaminase fold is interrupted by a conserved domain, which regulates the active site sterically via a large-scale conformational change and mechanistically via the Zn coordination geometry. Thus, we coined this novel domain 'gating domain' and the accompanying unusual metalloprotein regulation principle 'gated Zn-shutter'. An autoinhibited ground state and its activation by the presence of either ATP, GTP or the inhibitor tetrahydro uridine is consolidated by differential scanning fluorimetry as well as in vivo and in vitro RNA editing assays. In vivo, the framework of an active plant RNA editosome triggers the release of DYW autoinhibition to ensure a controlled and coordinated deamination likely playing a key role in mitochondrial and chloroplast homeostasis [4, 5].

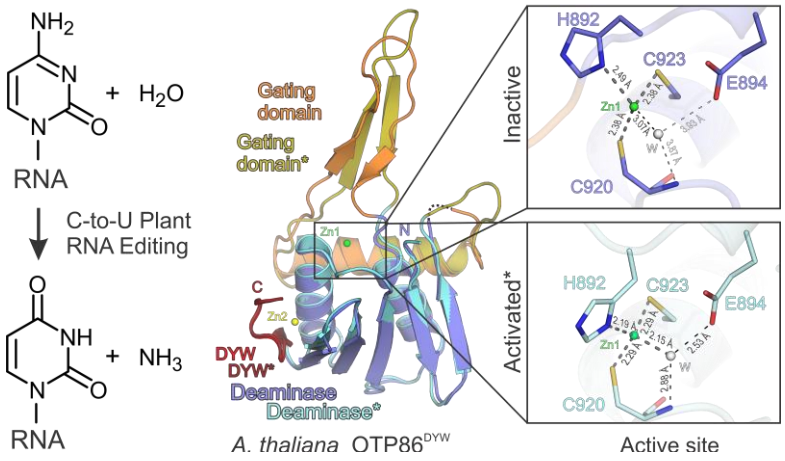

Fig. 1 An unusual regulation mechanism in plant RNA editing

[1] Hiesel R, Wissinger B, Schuster W and Brennicke A. RNA editing in plant mitochondria. Science 246, 1632–1634 (1989).
[2] Covello PS and Gray, MW. RNA editing in plant mitochondria. Nature 341, 662–666 (1989).
[3] Small ID, Schallenberg-Rüdinger M, Takenaka M, Mireau H and Ostersetzer-Biran O. Plant organellar RNA editing: what 30 years of research has revealed. Plant J. 101, 1040–1056 (2020).
[4] Takenaka M, Takenaka S, Barthel T, Frink B, Haag S, Verbitskiy D, Oldenkott B, Schallenberg-Rüdinger M, Feiler C, Weiss MS, Palm GJ and Weber G. DYW domain structures imply an unusual regulation mechanism in plant organellar RNA editing catalysis. Nature Catalysis 4, 510–522 (2021).
[5] Haag S, Schindler M, Berndt L, Brennicke A, Takenaka M and Weber G. Crystal structures of the *Arabidopsis thaliana* organellar RNA editing factors MORF1 and MORF9. NAR 45, 4915–4928 (2017).