Cyclophilin 38 – a plant immunophilin with a great hidden secret

A/P Kunchithapadam Swaminathan and his former graduate student Dr. Dileep Vasudevan (who has recently joined as Assistant Professor, Institute of Life Sciences, Bhubaneshwar, India), in collaboration with Prof. Sheng Luan at University of California, Berkeley, USA, solved the crystal structure of *Arabidopsis thaliana* Cyclophilin38 and revealed a hitherto uncharacterized immunophilin fold and a possible autoinhibitory mechanism. They published their results in **Plant Cell**, 24, 2666-74, 2012.

When humans undergo organ transplantation, they are given immune suppressor drugs, like FK506 and cyclosporin A. These drugs bid to their corresponding target immunophilin proteins, namely FK506 binding protein (FKBP) and cyclophilins, respectively. When immunophilins were discovered in the 1990s in plants, their functional roles were elusive. A/P Swaminathan and his then graduate student Dr. Gayathri Gopalan solved the first plant FKBP structure (Gopalan et al., **PNAS**, 101, 13945-13950, 2004) and elucidated the mechanism of their peptidyl-prolyl isomerase (PPIase) activity.

In this first plant cyclophilin CYP38 structure, A/P Swaminathan has shown a previously uncharacterized mode of auto-inhibition through inrta-molecular interactions and why CYP38 is not an active PPIase. Cyclophilin38 (CYP38) is one of the highly divergent cyclophilins from *Arabidopsis thaliana*. They solved the crystal structure of the At-CYP38 protein (residues 83-437 of 437 aa), at 2.39 Å resolution. The structure reveals two distinct domains: an N-terminal helical bundle and a C-terminal cyclophilin β-barrel, connected by an acidic loop. Two N-terminal β-strands become part of the C-terminal cyclophilin β-barrel, thereby making a previously undiscovered domain organization, Fig. 1.

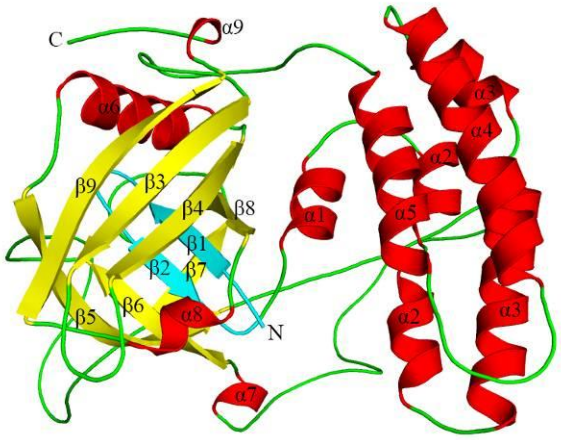

Figure 1. **The overall structure of At-CYP38**. A-helices are shown in red, β-strands in yellow, loops in green, the two N-terminal β-strands that form part of the C-terminal β-barrel shown in cyan.

The N-terminal helical domain is closely packed together with the putative C-terminal cyclophilin domain and establishes a strong intramolecular interaction, thereby preventing the access of the cyclophilin domain to other proteins. This was further verified by protein-protein interaction assays using the yeast two-hybrid system, Fig. 2.

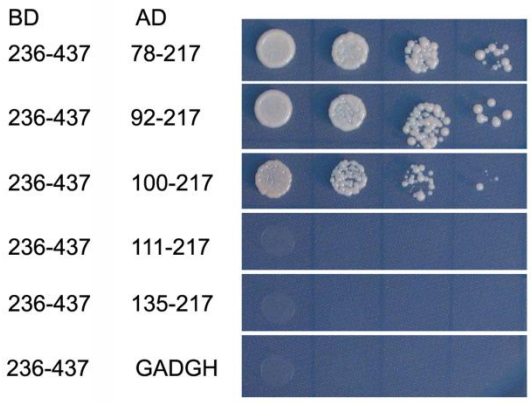

Figure 2. **Yeast two-hybrid analysis of domain interaction in CYP38**. Several N-terminal helical bundle truncation fragments and the CYP38 CYP domain were cloned into the binding domain (BD)- and activation domain (AD)-containing vectors, as labeled. The growth of yeast, when transformed with these constructs, on selection media indicates protein-protein interaction of the corresponding domains.

This study shows that CYP38 does not possess PPIase activity and identifies a possible interaction of CYP38 with the E-loop of CP47, a component of photosystem II. The interaction of CYP38 with the E-loop of CP47 is mediated through its cyclophilin domain.

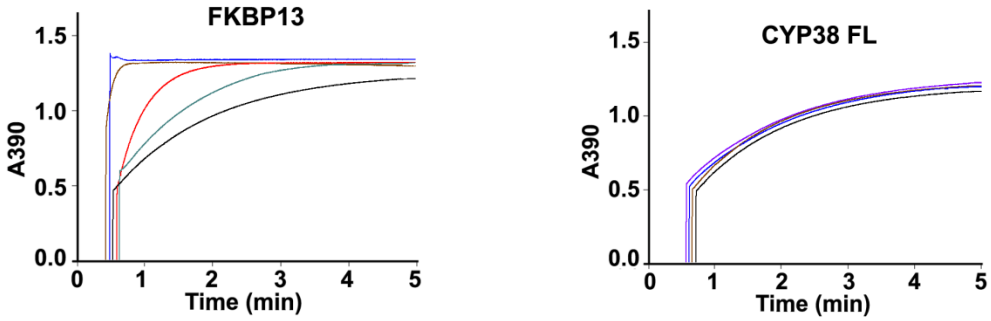

Figure 3. **Kinetic traces for ppiase activity measurement of FKBP13 and CYP38 FL**. In the panel for FKBP13, 1 ml reaction mixture contained 20 (blue), 2 (brown), and 0.2 (red), 0.02 (gray) protein, and 20 µg GST (black). In the panel for CYP38 FL, 1 ml reaction mixture contained 50 (purple), 20 (blue), 2 (brown) µg protein, and 20 µg GST (black).

Together, this study provides the structure of a plant cyclophilin and explains a possible mechanism for auto-inhibition of its function through an intramolecular interaction.